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(54) **Mycobacterial antigens expressed during latency**

(57) There is provided a therapeutic agent for combating mycobacterial infections, comprising an *M. tuberculosis* peptide having the sequence SEQ ID NO: 125, or a variant thereof having at least 70% amino acid identity therewith, or a fragment thereof having at least 10 amino acid residues, or a derivative thereof, wherein said variant, fragment or derivative has a common antigenic cross-reactivity to said isolated peptide; wherein the peptide is encoded by an *M. tuberculosis* gene the expres-

sion of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium.

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Description

[0001] The present invention relates to a method of identifying a gene in mycobacteria the expression of which is induced or up-regulated during mycobacterial latency, to the isolated peptide products, variants, derivatives or fragments thereof, to antibodies that bind to said peptides, variants, derivatives or fragments, to DNA and RNA vectors that express said peptides, variants, derivatives or fragments, to attenuated mycobacteria in which the activity of at least one of said induced or up-regulated genes has been modified, to vaccines against mycobacterial infections, and to methods of detecting the presence of a mycobacterial infection.

[0002] Many microorganisms are capable of forming intracellular infections. These include: infections caused by species of *Salmonella*, *Yersinia*, *Shigella*, *Campylobacter*, *Chlamydia* and *Mycobacteria*. Some of these infections are exclusively intracellular, others contain both intracellular and extracellular components. However, it is the intracellular survival cycle of bacterial infection which is suspected as a main supportive factor for disease progression.

[0003] Generally, these microorganisms do not circulate freely in the body, for example, in the bloodstream, and are often not amenable to drug treatment regimes. Where drugs are available, this problem has been exacerbated by the development of multiple drug resistant microorganisms.

[0004] A number of factors have contributed to the problem of microbial resistance. One is the accumulation of mutations over time and the subsequent horizontal and vertical transfer of the mutated genes to other organisms. Thus, for a given pathogen, entire classes of antibiotics have been rendered inactive. A further factor has been the absence of a new class of antibiotics in recent years. The emergence of multiple drug-resistant pathogenic bacteria represents a serious threat to public health and new forms of therapy are urgently required.

[0005] For similar reasons, vaccine therapies have not proved effective against such intracellular microorganisms. Also, increased systemic concentration of antibiotics to improve bioavailability within cells may result in severe side effects.

[0006] *Mycobacterium tuberculosis* (TB) and closely related species make up a small group of mycobacteria known as the *Mycobacterium tuberculosis* complex (MTC). This group comprises four species *M. tuberculosis*, *M. microti*, *M. bovis* and *M. africanum* which are the causative agent in the majority of tuberculosis (TB) cases throughout the world.

[0007] *M. tuberculosis* is responsible for more than three million deaths a year world-wide. Other mycobacteria are also pathogenic in man and animals, for example *M. avium* subsp. *paratuberculosis* which causes Johne's disease in ruminants, *M. bovis* which causes tuberculosis in cattle, *M. avium* and *M. intracellulare* which cause tuberculosis in immunocompromised patients (eg. AIDS patients, and bone marrow transplant patients) and *M. leprae* which causes leprosy in humans. Another important mycobacterial species is *M. vaccae*.

[0008] *M. tuberculosis* infects macrophage cells within the body. Soon after macrophage infection, most *M. tuberculosis* bacteria enter and replicate within cellular phagosome vesicles, where the bacteria are sequestered from host defences and extracellular factors.

[0009] It is the intracellular survival and multiplication or replication of bacterial infection which is suspected as a main supportive factor for mycobacterial disease progression.

[0010] A number of drug therapy regimens have been proposed for combatting *M. tuberculosis* infections, and currently combination therapy including the drug isoniazid has proved most effective. However, one problem with such treatment regimes is that they are long-term, and failure to complete such treatment can promote the development of multiple drug resistant microorganisms.

[0011] A further problem is that of providing an adequate bioavailability of the drug within the cells to be treated. Whilst it is possible to increase the systemic concentration of a drug (eg. by administering a higher dosage) this may result in severe side effects caused by the increased drug concentration.

[0012] The effectiveness of vaccine prevention against *M. tuberculosis* has varied widely. The current *M. tuberculosis* vaccine, BCG, is an attenuated strain of *M. bovis*. It is effective against severe complications of TB in children, but it varies greatly in its effectiveness in adults particularly across ethnic groups. BCG vaccination has been used to prevent tuberculous meningitis and helps prevent the spread of *M. tuberculosis* to extra-pulmonary sites, but does not prevent infection.

[0013] The limited efficacy of BCG and the global prevalence of TB has led to an international effort to generate new, more effective vaccines. The current paradigm is that protection will be mediated by the stimulation of a Th1 immune response.

[0014] BCG vaccination in man was given orally when originally introduced, but that route was discontinued because of loss of viable BCG during gastric passage and of frequent cervical adenopathy. In experimental animal species, aerosol or intratracheal delivery of BCG has been achieved without adverse effects, but has varied in efficacy from superior protection than parenteral inoculation in primates, mice and guinea pigs to no apparent advantage over the subcutaneous route in other studies.

[0015] There is therefore a need for an improved and/or alternative vaccine or therapeutic agent for combatting mycobacterial infections.

[0016] An additional major problem associated with the control of mycobacterial infections, especially *M. tuberculosis*

infections, is the presence of a large reservoir of asymptomatic individuals infected with mycobacteria. Dormant mycobacteria are even more resistant to front-line drugs.

[0017] Infection with mycobacteria (eg. *M. tuberculosis*) rarely leads to active disease, and most individuals develop a latent infection which may persist for many years before reactivating to cause disease (Wayne, 1994). The current strategy for controlling such infection is early detection and treatment of patients with active disease. Whilst this is essential to avoid deaths and control transmission, it has no effect on eliminating the existing reservoir of infection or on preventing new cases of disease through reactivation.

[0018] Conventional mycobacterial vaccines, including BCG, protect against disease and not against infection. Ideally a new mycobacterial vaccine will impart sterile immunity, and a post-exposure vaccine capable of boosting the immune system to kill latent mycobacteria or prevent reactivation to active disease-causing microorganisms would also be valuable against latent infection.

Conventional detection of latent mycobacterial infection by skin testing may be compromised. For example, current TB detection methods based on tuberculin skin testing are compromised by BCG vaccination and by exposure to environmental mycobacteria.

[0019] New strategies are therefore required for more effective diagnosis, treatment and prevention of mycobacterial latent infection.

[0020] To develop specific strategies for addressing latent mycobacterial infection it is necessary to elucidate the physiological, biochemical and molecular properties of these microorganisms.

[0021] At present, there is no suitable *in vivo* model for studying mycobacterial latent infection and such a model is unlikely to provide sufficient microbial material to enable detailed analysis of the physiological and molecular changes that occur.

[0022] Studies to date have used either static cultures which allow tubercle bacilli to generate oxygen-depletion gradients and enter a non-replicating persistent state in the sediment layer, or agitated sealed liquid cultures (Wayne and Lin, 1982; Cunningham and Spreadbury, 1998; Wayne and Hayes, 1996). Transition to a non-replicating persistent state in these models coincides with a shift-down to glyoxylate metabolism, resistance to isoniazid and rifampicin and susceptibility to the anaerobic bactericidal action of metronidazole (Wayne and Hayes, 1996).

[0023] For example, a number of publications have described the analysis of mycobacterial gene and protein expression profiles following exposure of the mycobacteria to various environmental stimuli. These include Sherman, D.R. et al (2001) PNAS, vol. 98, no. 13, pp. 7534-7539; Hutter, B. (2000) FEMS Microbiol. Letts. 188, pp. 141-146; Michele, T.M. et al. (1999) Antimicrobial Agents and Chemotherapy, vol. 43, no. 2, pp. 216-225; Yuan, Y. et al. (1998) PNAS, vol. 95, pp. 9578-9583; Boon, C. et al (2001) J. Bacteriol., vol. 183, no. 8, pp. 2672-2676; Cunningham, A.F. et al (1998) J. Bacteriol., vol. 180, no. 4, pp. 801-808; Murugasu-Oei, B. et al (1999) Mol. Gen. Genet., vol. 262, pp. 677-682; and a number of patent publications such as WO99/24067, WO99/04005, WO97135611, and WO92/08484. The mycobacteria employed in these analyses have been grown in crude, batch systems, with the result that there is little or no control of the environmental stimuli to which the mycobacteria have been exposed. Accordingly, the bacteria experience a large number of complex, interactive environmental stimuli, some of which may have rapid and transient effects in terms of gene and protein expression.

[0024] Such studies are poorly defined and controlled, and experiments relying on self-generated oxygen-depletion gradients have yielded inconsistent results. In addition, the described studies have been conducted over a relatively short duration in terms of post-inoculation growth, in many cases up to approximately 2 weeks post-inoculation, with the result that the cultured bacteria are exposed to environmental stimuli associated with the mid to late exponential phase, and/or the early stationary phase.

[0025] In view of the above, there is a need for a defined and controlled model for studying mycobacterial (eg. TB) persistence which simulates key features of the *in vivo* environment.

[0026] According to a first aspect of the present invention there is provided an isolated mycobacterial peptide, or a fragment or derivative or variant of said peptide, wherein the peptide is encoded by a mycobacterial gene the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of a mycobacterium for at least 20 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium.

[0027] Latency is synonymous with persistence. These terms describe a reversible state of low metabolic activity in which mycobacterial cells can survive for extended periods without cell division.

[0028] In contrast to the various prior art analyses, the present invention is concerned with the induction or up-regulation of mycobacterial genes (and the corresponding gene products) during long term latency conditions rather than during the onset of latency (ie. late exponential phase, or early stationary phase).

[0029] The preferred culture method of the present invention is that of batch fermenter culture. This method permits careful monitoring and control of growth culture parameters such as pH, temperature, available nutrients, and dissolved oxygen tension (DOT). In particular, temperature and DOT may be strictly controlled. In contrast, careful monitoring and

control is not possible with convention, crude batch culture systems, with the result that mycobacteria cultured by such systems are exposed to a multiplicity of complex, interactive environmental stimuli, some of which may have rapid and transient effects in terms of gene and protein expression. Thus, the batch fermenter system of the present invention allows relatively careful control of environmental stimuli so that a mycobacterial response to a particular stimulus (eg. nutrient starvation) can be analysed in relative isolation from other environmental stimuli that may otherwise obscure or modify the particular mycobacterial response of interest.

[0030] In use of the present method it is possible to ensure that the principal latency induction parameter employed is starvation of carbon, and preferably the starvation of carbon and energy. This means that the accidental induction or up-regulation of genes that are solely responsive to other environmental switches may be substantially prevented. Accordingly, false-positive identification of genes that are induced or up-regulated under conditions unrelated to carbon starvation and/or energy limitation may be substantially avoided.

[0031] The term "nutrient-starving" in the context of the present invention means that the concentration of the primary carbon, and preferably the primary energy source, is insufficient to support growth of the mycobacteria. "Nutrient-starving" is a term associated with an established mid to late stationary phase of a batch culture growth curve. Under such conditions the mycobacteria are metabolically stressed, rather than simply reduced in growth rate.

[0032] In more detail, exponential growth is that period of growth which is associated with a logarithmic increase in mycobacterial cell mass (also known as the "log" phase) in which the bacteria are multiplying at a maximum specific growth rate for the prevailing culture conditions. During this period of growth the concentrations of essential nutrients diminish and those of end products increase. However, once the primary carbon and/or primary energy source falls to below a critical level, it is no longer possible for all of the mycobacterial cells within the culture to obtain sufficient carbon and/or energy needed to support optimal cellular function and cell division. Once this occurs, exponential growth slows and the mycobacteria enter stationary phase. Thereafter, the mycobacteria become nutrient starved, and enter latency. It is this latent state in the growth phase, rather than the late exponential phase or early stationary phase, with which the present invention is concerned.

[0033] Carbon starvation refers to a growth state in which the concentration of exogenous carbon is insufficient to enable the bacteria to grow and or replicate. However, when in this state, there may be other energy sources (eg. endogenous reserves, secondary metabolites) that are available to maintain essential cellular functions and viability without supporting growth. Thus, carbon starvation is associated with a mid or late stationary phase condition in which the exogenous carbon source has become depleted and bacterial growth has substantially ceased. In terms of a batch fermenter culture of mycobacteria, this typically occurs at 20 days (or later) post inoculation.

[0034] The onset of stationary phase *vis-a-vis* the time of inoculation will depend on a number of factors such as the particular mycobacterial species/strain, the composition of the culture media (eg. the particular primary carbon and energy source), and the physical culture parameters employed.

[0035] However, as a guide, the end of exponential phase and the onset of stationary phase generally corresponds to that point in the growth phase associated with the maximum number of viable counts of mycobacteria.

[0036] In use of the present invention, the exponential phase mycobacterial cells are harvested from the culture vessel at a point in the growth phase before the maximum number of total viable counts has been achieved. This point in the growth phase may be mimicked under continuous culture conditions employing a steady state growth rate approximating $\mu_{\max}$ and providing a generation time of approximately 18-24 hours. In a preferred embodiment, the exponential phase mycobacterial cells are harvested when a value of between 2 and 0.5 (more preferably between 1 and 0.5) log units of viable counts per ml of culture medium less than the maximum number of viable counts per ml of culture medium has been achieved. Thus, the "exponential" phase cells are generally harvested during mid-log phase.

[0037] For example, if the maximum viable count value is 1×10^{10} per ml, then the "exponential" phase cells would be preferably harvested once a value of between 1×10^8 and $1 \times 10^{9.5}$ (more preferably between 1×10^9 and $1 \times 10^{9.5}$) viable counts per ml has been achieved. In the case of *M. tuberculosis*, this would be approximately 3-10, preferably 4-7 days post-inoculation.

[0038] In use of the present invention, the nutrient-starved, batch fermenter cultured mycobacterial cells are harvested from the culture vessel at a point in the growth phase after the maximum number of total viable counts has been achieved. This point in the growth phase may be mimicked under continuous culture conditions supporting a generation time of at least 3 days. In a preferred embodiment, the stationary phase mycobacterial cells are harvested when the viable counts per ml of culture medium has fallen by at least 0.5, preferably at least 1, more preferably at least 2 log units less than the maximum number of viable counts per ml of culture medium. Thus, the nutrient-starved cells are generally harvested during mid- to late-stationary phase.

[0039] For example, if the maximum viable count value is 1×10^{10} per ml, then the stationary phase cells would be preferably harvested once the viable count number had fallen to a value of at least $1 \times 10^{9.5}$, preferably at least 1×10^9 , more preferably at least 1×10^8 viable counts per ml. In the case of *M. tuberculosis*, this would be approximately at least day 20, preferably at least day 30, typically day 40-50 post-inoculation. Longer post-inoculation harvesting times of at least 100 days, even at least 150 days may be employed. For mycobacteria generally, the mid to late stationary phase

cells are preferably harvested at least 20 days, preferably at least 30 days, more preferably at least 40 days post-inoculation.

[0040] Suitable media for culturing mycobacteria are described in Wayne, L.G. (1994) [in *Tuberculosis: Pathogenesis, Protection, and Control* published by the American Society for Microbiology, pp. 73-83]. These include Middlebrook 7H9 Medium [see Barker, L.P., et al. (1998) *Molec. Microbiol.*, vol. 29(5), pp. 1167-1177], and WO00152139 in the name of the present Applicant.

[0041] In use of the batch fermenter culture method, the starting concentration of the primary carbon source (and preferably the primary energy source) is at least 0.5, preferably at least 1 g/l of culture medium. Such concentrations are considered to be not nutrient-starving. Conversely, "nutrient-starving" conditions are associated with a primary carbon and energy source concentration of less than 0.5, preferably less than 0.2, and more preferably less than 0.1 g/l of culture medium. The preferred carbon and energy source is glycerol.

[0042] In a preferred embodiment, the starting concentration of glycerol is at least 1, preferably 1-3, more preferably approximately 2 g/l of culture medium. The onset of "nutrient-starving" conditions is associated with a concentration of less than 0.2, preferably less than 0.1 g/l of culture medium.

[0043] Other primary carbon and energy sources may be employed such as glucose, pyruvate, and fatty acids (eg. palmitate, and butyrate). These sources may be employed at substantially the same concentrations as for glycerol.

[0044] The pH of the culture medium is preferably maintained between pH 6 and 8, more preferably between pH 6.5 and 7.5, most preferably at about pH 6.9.

[0045] In one embodiment, the dissolved oxygen tension (DOT) is maintained throughout the culture process at at least 40 % air saturation, more preferably between 50 and 70 % air saturation, most preferably at 50% air saturation.

[0046] The dissolved oxygen tension parameter is calculated by means of an oxygen electrode and conventional laboratory techniques. Thus, 100 % air saturation corresponds to a solution that is saturated with air, whereas 0% corresponds to a solution that has been thoroughly purged with an inert gas such as nitrogen. Calibration is performed under standard atmospheric pressure conditions and measured at 37 °C, and with conventional air comprising approximately 21 % oxygen.

[0047] In another embodiment of the present invention, latency may be induced by a combination of carbon and/or energy source starvation, and a low DOT.

[0048] In a preferred embodiment, the DOT is maintained at at least 40 % air saturation, more preferably between 50 and 70 % air saturation, until the mycobacterial culture has entered early-mid log phase. The DOT may be then lowered so as to become limiting, for example in increments over a 5 or 6 day period, and the culture maintained at a DOT of 0-10, preferably at a DOT of approximately 5 % until the stationary phase cells are harvested.

[0049] The carbon and energy starvation, and optional low oxygen tension latency induction conditions of the present invention are culture conditions that are conducive for a mycobacterium to express at least one gene which would be normally expressed *in vivo* during latency of the mycobacterium's natural target environment which is believed to involve a low carbon and energy, and low oxygen environment.

[0050] The mycobacterium is selected from the species *M. phlei*, *M. smegmatis*, *M. africanum*, *M. caneti*, *M. fortuitum*, *M. marinum*, *M. ulcerans*, *M. tuberculosis*, *M. bovis*, *M. microti*, *M. avium*, *M. para tuberculosis*, *M. leprae*, *M. leprae-murium*, *M. intracellulare*, *M. scrofulaceum*, *M. xenopi*, *M. genavense*, *M. kansasii*, *M. simiae*, *M. szulgai*, *M. haemophilum*, *M. asiaticum*, *M. malmoense*, *M. vaccae* and *M. shimoidei*. Of particular interest are members of the MTC, preferably *M. tuberculosis*.

[0051] In use, it is preferred that those genes (ie. as represented by cDNAs in the detection assay) which are up-regulated by at least 1.5-fold under stationary phase conditions *vis-a-vis* exponential phase conditions are selected. In more preferred embodiments, the corresponding up-regulation selection criterion is at least 2-fold, more preferably 3-fold, most preferably 4-fold. In further embodiments up-regulation levels of at least 10-fold, preferably 50-fold may be employed.

[0052] The term peptide throughout this specification is synonymous with protein.

[0053] Use of mycobacterial peptide compositions, which peptides are associated with mycobacterial latency, provide excellent vaccine candidates for targeting latent mycobacteria in asymptomatic patients infected with mycobacteria.

[0054] The terms "isolated," "substantially pure," and "substantially homogenous" are used interchangeably to describe a peptide which has been separated from components which naturally accompany it. A peptide is substantially pure when at least about 60 to 75% of a sample exhibits a single peptide sequence. A substantially pure peptide will typically comprise about 60 to 90% w/w of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Peptide purity or homogeneity may be indicated by, for example, polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel. Alternatively, higher resolution may be provided by using, for example, HPLC. A peptide is considered to be isolated when it is separated from the contaminants which accompany it in its natural state. Thus, a peptide which is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. The present invention provides peptides which may be purified from mycobacteria as well as from other

types of cells transformed with recombinant nucleic acids encoding these peptides. If desirable, the amino acid sequence of the proteins of the present invention may be determined by protein sequencing methods.

[0055] The terms "peptide", "oligopeptide", "polypeptide", and "protein" are used interchangeably and do not refer to a specific length of the product. These terms embrace post-translational modifications such as glycosylation, acetylation, and phosphorylation.

[0056] The term "fragment" means a peptide having at least five, preferably at least ten, more preferably at least twenty, and most preferably at least thirty-five amino acid residues of the peptide which is the gene product of the induced or up-regulated gene in question. The fragment preferably includes an epitope of the gene product in question.

[0057] The term "variant" means a peptide or peptide "fragment" having at least seventy, preferably at least eighty, more preferably at least ninety percent amino acid sequence homology with the peptide that is the gene product of the induced or up-regulated gene in question. An example of a "variant" is a peptide or peptide fragment of an induced/up-regulated gene which contains one or more analogues of an amino acid (eg. an unnatural amino acid), or a substituted linkage. The terms "homology" and "identity" are considered synonymous in this specification. In a further embodiment, a "variant" may be a mimic of the peptide or peptide fragment, which mimic reproduces at least one epitope of the peptide or peptide fragment. The mimic may be, for example, a nucleic acid mimic, preferably a DNA mimic.

[0058] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences may be compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequent coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percentage sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0059] Optimal alignment of sequences for comparison may be conducted, for example, by the local homology alignment algorithm of Smith and Waterman [Adv. Appl. Math. 2: 484 (1981)], by the algorithm of Needleman & Wunsch [J. Mol. Biol. 48: 443 (1970)] by the search for similarity method of Pearson & Lipman [Proc. Nat'l. Acad. Sci. USA 85: 2444 (1988)], by computer implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA - Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705), or by visual inspection [see Current Protocols in Molecular Biology, F.M. Ausbel et al, eds, Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (1995 Supplement) Ausubel].

[0060] Examples of algorithms suitable for determining percent sequence similarity are the BLAST and BLAST 2.0 algorithms [see Altschul (1990) J. Mol. Biol. 215: pp. 403-410; and "<http://www.ncbi.nlm.nih.gov/>" of the National Center for Biotechnology Information].

[0061] In a preferred homology comparison, the identity exists over a region of the sequences that is at least 10 amino acid residues in length.

[0062] The term "derivative" means a peptide comprising the peptide (or fragment, or variant thereof) which is the gene product of the induced or up-regulated gene in question. Thus, a derivative may include the peptide in question, and a further peptide sequence which may introduce one or more additional epitopes. The further peptide sequence should preferably not interfere with the basic folding and thus conformational structure of the peptide in question. Examples of a "derivative" are a fusion protein, a conjugate, and a graft. Thus, two or more peptides (or fragments, or variants) may be joined together to form a derivative. Alternatively, a peptide (or fragment, or variant) may be joined to an unrelated molecule (eg. a peptide). Derivatives may be chemically synthesized, but will be typically prepared by recombinant nucleic acid methods. Additional components such as lipid, and/or polysaccharide, and/or polyketide components may be included.

[0063] All of the molecules "fragment", "variant" and "derivative" have a common antigenic cross-reactivity and/or substantially the same *in vivo* biological activity as the gene product of the induced or up-regulated gene in question from which they are derived. For example, an antibody capable of binding to a fragment, variant or derivative would be also capable of binding to the gene product of the induced or up-regulated gene in question. It is a preferred feature that the fragment, variant and derivative each possess the active site of the peptide which is the induced or up-regulated peptide in question. Alternatively, all of the above embodiments of a peptide of the present invention share a common ability to induce a "recall response" of a T-lymphocyte which has been previously exposed to an antigenic component of a mycobacterial infection.

[0064] In a preferred embodiment, the peptide is selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279 and 281.

[0065] According to a second aspect of the invention there is provided a method of identifying a mycobacterial gene

the expression of which is induced or up-regulated during mycobacterial latency, said method comprising:-

5 culturing a first mycobacterium under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of the first mycobacterium for at least 20 days post-inoculation;
 culturing a second mycobacterium under culture conditions that are not nutrient-starving and which support exponential growth of the second mycobacterium;
 obtaining first and second mRNA populations from said first and second mycobacteria respectively, wherein said first mRNA population is obtained from the first mycobacterium which has been cultured under nutrient-starving conditions obtainable by batch fermentation of the first mycobacterium for at least 20 days post-inoculation, and wherein said second mRNA is obtained from the second mycobacterium which has been cultured under conditions that are not nutrient-starving and which support exponential growth of said second mycobacterium;
 10 preparing first and second cDNA populations from said first and second mRNA populations respectively, during which cDNA preparation a detectable label is introduced into the cDNA molecules of the first and second cDNA populations;
 isolating corresponding first and second cDNA molecules from the first and second cDNA populations, respectively; comparing relative amounts of label or corresponding signal emitted from the label present in the isolated first and second cDNA molecules;
 identifying a greater amount of label or signal provided by the isolated first cDNA molecule than that provided by the isolated second cDNA molecule; and
 20 identifying the first cDNA and the corresponding mycobacterial gene which is induced or up-regulated during mycobacterial latency.

[0066] Reference to gene throughout this specification embraces open reading frames (ORFs).

25 [0067] The various embodiments described for the first aspect of the present invention apply equally to the second and subsequent aspects of the present invention.

[0068] The term "corresponding first and second cDNA molecules from the first and second cDNA populations" refers to cDNAs having substantially the same nucleotide sequence. Thus, by isolating the cDNA copies relating to a given gene under each culture condition (ie. exponential phase, and stationary phase), it is possible to quantify the relative copy number of cDNA for that gene for each culture condition. Since each cDNA copy has been produced from an mRNA molecule, the cDNA copy number reflects the corresponding mRNA copy number for each culture condition, and thus it is possible to identify induced or up-regulated genes.

30 [0069] In one embodiment, the first and second cDNA molecules are isolated from the corresponding first and second cDNA populations by hybridisation to an array containing immobilised DNA sequences that are representative of each known gene (or ORF) within a particular mycobacterial species genome. Thus, a first cDNA may be considered "corresponding" to a second cDNA if both cDNAs hybridise to the same immobilised DNA sequence.

[0070] In another embodiment, the first and second cDNAs are prepared by incorporation of a fluorescent label. The first and second cDNAs may incorporate labels which fluoresce at different wavelengths, thereby permitting dual fluorescence and simultaneous detection of two cDNA samples.

40 [0071] The type of label employed naturally determines how the output of the detection method is read. When using fluorescent labels, a confocal laser scanner is preferably employed.

[0072] According to one embodiment, fluorescently labelled cDNA sequences from stationary and exponential phase cultured systems were allowed to hybridise with a whole mycobacterial genome array. The first cDNA population was labelled with fluorescent label A, and the second cDNA population was labelled with fluorescent label B. The array was scanned at two different wavelengths corresponding to the excitable maxima of each dye and the intensity of the emitted light was recorded. Multiple arrays were preferably prepared for each cDNA and a mean intensity value was calculated across the two cDNA populations for each spot with each dye, against which relative induction or up-regulation was quantified.

50 [0073] In addition to the above mRNA isolation and cDNA preparation and labelling, genomic DNA may be isolated from the first and second mycobacteria. Thus, in a preferred embodiment, labelled DNA is also prepared from the isolated DNA. The labelled DNA may be then included on each array as a control.

[0074] According to a third aspect of the present invention, there is provided an inhibitor of a mycobacterial peptide, wherein the peptide is encoded by a gene the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of a mycobacterium for at least 20 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium, wherein the inhibitor is capable of preventing or inhibiting the mycobacterial peptide, from exerting its native biological effect.

55 [0075] Such inhibitors may be employed to prevent the onset of, or to cause a break in the period of mycobacterial

latency (ie. induce re-activation). In this respect, mycobacteria are more susceptible to treatment regimens when in a non-latent state, and the combined use of drugs to kill latent mycobacteria (eg. TB) would significantly reduce the incidence of mycobacteria by targeting the reservoir for new disease and would thereby help reduce the problem of emerging drug-resistant strains.

[0076] The inhibitor may be a peptide, carbohydrate, synthetic molecule, or an analogue thereof. Inhibition of the mycobacterial peptide may be effected at the nucleic acid level (ie. DNA, or RNA), or at the peptide level. Thus, the inhibitor may act directly on the peptide. Alternatively, the inhibitor may act indirectly on the peptide by, for example, causing inactivation of the induced or up-regulated mycobacterial gene.

[0077] In preferred embodiments, the inhibitor is capable of inhibiting one or more of the following:- 2-nitropropane dioxygenase, acetyltransferase, oxidoreductase, transcriptional regulator, acyl transferase, UDP-glucose dehydrogenase, phosphoribosylglycinamide formyltransferase, 1,4-dihydroxy-2-naphthoate octaprenyl, gmc-type oxidoreductase, 3-hydroxyisobutyrate dehydrogenase, methylmalonate semialdehyde dehydrogenase, dehydrogenase, mercuric reductase, glutathione reductase, dihydrolipoamide, transposase, proline iminopeptidase, prolyl aminopeptidase, quinolone efflux pump, glycine betaine transporter, phosphatidylethanolamine N-methyltransferase, chalcone synthase 2, sulfotransferase, glycosyl transferase, fumarate reductase flavoprotein, 8-amino-7-oxononanoate synthase, aminotransferase class-II pyridoxal-phosphate, bacteriophage HK97 prohead protease, penicillin-binding protein, fatty acyl-CoA racemase, nitrilotriacetate monooxygenase, histidine kinase response regulator, peptidase, LysR transcription regulator, excisionase, ornithine aminotransferase, malate oxidoreductase, thiosulphate binding protein, enoyl-CoA hydratase, acyl-CoA synthetase, methyltransferase, siroheme synthase, permease, glutaryl 7-aca acylase, sn-glycerol-3-phosphate transport system permease, enoyl-CoA hydratase/isomerase, acyl-CoA dehydrogenase, esterase, lipase, cytidine deaminase, crotonase, lipid-transfer protein, acetyl-CoA C-acetyltransferase, aminotransferase, hydrolase, and 2-amino-4-hydroxy-6-hydroxymethylidihydropterine pyrophosphokinase.

[0078] In a further embodiment, the inhibitor may be an antibiotic capable of targeting the induced or up-regulated mycobacterial gene identifiable by the present invention, or the gene product thereof. The antibiotic is preferably specific for the gene and/or gene product.

[0079] In a further embodiment, the inhibitor may act on a gene or gene product the latter of which interacts with the induced or up-regulated gene. Alternatively, the inhibitor may act on a gene or gene product thereof upon which the gene product of the induced or up-regulated gene acts.

[0080] Inhibitors of the present invention may be prepared utilizing the sequence information of provided herein. For example, this may be performed by overexpressing the peptide, purifying the peptide, and then performing X-ray crystallography on the purified peptide to obtain its molecular structure. Next, compounds are created which have similar molecular structures to all or portions of the polypeptide or its substrate. The compounds may be then combined with the peptide and attached thereto so as to block one or more of its biological activities.

[0081] Also included within the invention are isolated or recombinant polynucleotides that bind to the regions of the mycobacterial chromosome containing sequences that are associated with induction/up-regulation under low oxygen tension (ie. virulence), including antisense and triplex-forming polynucleotides. As used herein, the term "binding" refers to an interaction or complexation between an oligonucleotide and a target nucleotide sequence, mediated through hydrogen bonding or other molecular forces. The term "binding" more specifically refers to two types of internucleotide binding mediated through base-base hydrogen bonding. The first type of binding is "Watson-Crick-type" binding interactions in which adenine-thymine (or adenine-uracil) and guanine-cytosine base-pairs are formed through hydrogen bonding between the bases. An example of this type of binding is the binding traditionally associated with the DNA double helix and in RNA-DNA hybrids; this type of binding is normally detected by hybridization procedures. The second type of binding is "triplex binding". In general, triplex binding refers to any type of base-base hydrogen bonding of a third polynucleotide strand with a duplex DNA (or DNA-RNA hybrid) that is already paired in a Watson-Crick manner.

[0082] In a preferred embodiment, the inhibitor may be an antisense nucleic acid sequence which is complementary to at least part of the inducible or up-regulatable gene.

[0083] The inhibitor, when in the form of a nucleic acid sequence, in use, comprises at least 15 nucleotides, preferably at least 20 nucleotides, more preferably at least 30 nucleotides, and most preferably at least 50 nucleotides.

[0084] According to a fourth aspect of the invention, there is provided an antibody that binds to a peptide encoded by a gene, or to a fragment or variant or derivative of said peptide, the expression of which gene is induced or up-regulated during culture of a mycobacterium under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of a mycobacterium for at least 20 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium.

[0085] The antibody preferably has specificity for the peptide in question, and following binding thereto may initiate coating of the mycobacterium. Coating of the bacterium preferably leads to opsonization thereof. This, in turn, leads to the bacterium being destroyed. It is preferred that the antibody is specific for the mycobacterium (eg. species and/or strain) which is to be targeted.

In use, the antibody is preferably embodied in an isolated form.

[0086] Opsonization by antibodies may influence cellular entry and spread of mycobacteria in phagocytic and non-phagocytic cells by preventing or modulating receptor-mediated entry and replication in macrophages.

[0087] The peptides, fragments, variants or derivatives of the present invention may be used to produce antibodies, including polyclonal and monoclonal antibodies. If polyclonal antibodies are desired, a selected mammal (eg. mouse, rabbit, goat, horse, etc.) is immunized with an immunogenic polypeptide. Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to a desired mycobacterial epitope contains antibodies to other antigens, the polyclonal antibodies may be purified by immunoaffinity chromatography. Alternatively, general methodology for making monoclonal antibodies by hybridomas involving, for example, preparation of immortal antibody-producing cell lines by cell fusion, or other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus may be employed.

[0088] The antibody employed in this aspect of the invention may belong to any antibody isotype family, or may be a derivative or mimic thereof. Reference to antibody throughout this specification embraces recombinantly produced antibody, and any part of an antibody which is capable of binding to a mycobacterial antigen.

[0089] In one embodiment the antibody belongs to the IgG, IgM or IgA isotype families.

[0090] In a preferred embodiment, the antibody belongs to the IgA isotype family. Reference to the IgA isotype throughout this specification includes the secretory form of this antibody (ie. slgA). The secretory component (SC) of slgA may be added *in vitro* or *in vivo*. In the latter case, the use of a patient's natural SC labelling machinery may be employed.

[0091] In one embodiment, the antibody may be raised against a peptide from a member of the MTC, preferably against *M. tuberculosis*.

[0092] In a preferred embodiment, the antibody is capable of binding to a peptide selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281 and a fragment, variant, and derivative of said SEQ IDs.

[0093] In a further embodiment, the antigen is an exposed component of a mycobacterial bacillus. In another embodiment, the antigen is a cell surface component of a mycobacterial bacillus.

[0094] The antibody of the present invention may be polyclonal, but is preferably monoclonal.

[0095] Without being bound by any theory, it is possible that following mycobacterial infection of a macrophage, the macrophage is killed and the bacilli are released. It is at this stage that the mycobacteria are considered to be most vulnerable to antibody attack. Thus, it is possible that the antibodies of the present invention act on released bacilli following macrophage death, and thereby exert a post-infection effect.

[0096] It is possible that the passive protection aspect (ie. delivery of antibodies) of the present invention is facilitated by enhanced accessibility of the antibodies of the present invention to antigens on mycobacterial bacilli harboured by the infected macrophages. Indeed, *acr* expression is low during logarithmic growth, but increases at the stationary or oxygen limiting stage, and particularly in organisms which replicate within macrophages. As *acr* expression appears to be necessary for mycobacterial infectivity, it is possible that antibody binding may block macrophage infection by steric hindrance or disruption of its oligomeric structure. Thus, antibodies acting on mycobacterial bacilli released from killed, infected macrophages may interfere with the spread of re-infection to fresh macrophages. This hypothesis involves a synergistic action between antibodies and cytotoxic T cells, acting early after infection, eg. $\gamma\delta$ and NK T cells, but could later involve also CD8 and CD4 cytotoxic T cells.

[0097] According to a fifth aspect of the invention, there is provided an attenuated mycobacterium in which a gene has been modified thereby rendering the mycobacterium substantially non-pathogenic, wherein said gene is a gene the expression of which is induced or up-regulated during culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of a mycobacterium for at least 20 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium.

[0098] The modification preferably inactivates the gene in question, and preferably renders the mycobacterium substantially non-pathogenic.

[0099] The term "modified" refers to any genetic manipulation such as a nucleic acid or nucleic acid sequence replacement, a deletion, or an insertion which renders the mycobacterium substantially reduced in ability to persist in a latent state. In one embodiment the entire inducible or up-regulatable gene may be deleted.

[0100] In a preferred embodiment, gene to be modified has a wild-type coding sequence selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156,

158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280 and 282.

[0101] It will be appreciated that the above wild-type sequences may include minor variations depending on the Database employed. The term "wild-type" indicates that the sequence in question exists as a coding sequence in nature.

[0102] According to a sixth aspect of the invention, there is provided an attenuated microbial carrier, comprising a peptide encoded by a gene, or a fragment or variant or derivative of said peptide, the expression of which gene is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of a mycobacterium for at least 20 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium.

[0103] In use, the peptide (or fragment, variant or derivative) is either at least partially exposed at the surface of the carrier, or the carrier becomes degraded *in vivo* so that at least part of the peptide (or fragment, variant or derivative) is otherwise exposed to a host's immune system.

[0104] In a preferred embodiment, the attenuated microbial carrier is attenuated salmonella, attenuated vaccinia virus, attenuated fowlpox virus, or attenuated *M. bovis* (eg. BCG strain).

[0105] In a preferred embodiment, the peptide is selected from the group consisting of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279 and 281.

[0106] According to a seventh aspect of the invention, there is provided a DNA plasmid comprising a promoter, a polyadenylation signal, and a DNA sequence that is the coding sequence of a mycobacterial gene or a fragment or variant or derivative of said coding sequence, the expression of which gene is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of a mycobacterium for at least 20 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium, wherein the promoter and polyadenylation signal are operably linked to the DNA sequence.

[0107] The term DNA "fragment" used in this invention will usually comprise at least about 5 codons (15 nucleotides), more usually at least about 7 to 15 codons, and most preferably at least about 35 codons. This number of nucleotides is usually about the minimal length required for a successful probe that would hybridize specifically with such a sequence.

[0108] In preferred embodiments, the DNA "fragment" has a nucleotide length which is at least 50%, preferably at least 70%, and more preferably at least 80% that of the coding sequence of the corresponding induced/up-regulated gene.

[0109] The term DNA "variant" means a DNA sequence that has substantial homology or substantial similarity to the coding sequence (or a fragment thereof) of an induced/up-regulated gene. A nucleic acid or fragment thereof is "substantially homologous" (or "substantially similar") to another if, when optimally aligned (with appropriate nucleotide insertions or deletions) with the other nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least about 95 to 98% of the nucleotide bases. Homology determination is performed as described *supra* for peptides.

[0110] Alternatively, a DNA "variant" is substantially homologous (or substantially similar) with the coding sequence (or a fragment thereof) of an induced/up-regulated gene when they are capable of hybridizing under selective hybridization conditions. Selectivity of hybridization exists when hybridization occurs which is substantially more selective than total lack of specificity. Typically, selective hybridization will occur when there is at least about 65% homology over a stretch of at least about 14 nucleotides, preferably at least about 70%, more preferably at least about 75%, and most preferably at least about 90%. See, Kanehisa (1984) Nuc. Acids Res. 12:203-213. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of at least about 17 nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides.

[0111] Nucleic acid hybridization will be affected by such conditions as salt concentration (eg. NaCl), temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. Stringent temperature conditions are preferably employed, and generally include temperatures in excess of 30°C, typically in excess of 37°C and preferably in excess of 45°C. Stringent salt conditions will ordinarily be less than 1000 mM, typically less than 500 mM, and preferably less than 200 mM. The pH is typically between 7.0 and 8.3. However, the combination of parameters is much more important than the measure of any single parameter. See, eg., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370.

[0112] The term DNA "derivative" means a DNA polynucleotide which comprises a DNA sequence (or a fragment, or variant thereof) corresponding to the coding sequence of the induced/up-regulated gene and an additional DNA sequence which is not naturally associated with the DNA sequence corresponding to the coding sequence. The comments on peptide derivative *supra* also apply to DNA "derivative". A "derivative" may, for example, include two or more coding sequences of a mycobacterial operon that is induced during nutrient-starvation. Thus, depending on the presence or absence of a non-coding region between the coding sequences, the expression product/s of such a "derivative" may be a fusion protein, or separate peptide products encoded by the individual coding regions.

[0113] The above terms DNA "fragment", "variant", and "derivative" have in common with each other that the resulting peptide products have cross-reactive antigenic properties which are substantially the same as those of the corresponding wild-type peptide. Preferably all of the peptide products of the above DNA molecule embodiments of the present invention bind to an antibody which also binds to the wild-type peptide. Alternatively, all of the above peptide products are capable of inducing a "recall response" of a T lymphocyte which has been previously exposed to an antigenic component of a mycobacterial infection.

[0114] The promoter and polyadenylation signal are preferably selected so as to ensure that the gene is expressed in a eukaryotic cell. Strong promoters and polyadenylation signals are preferred.

[0115] In a related aspect, the present invention provides an isolated RNA molecule which is encoded by a DNA sequence of the present invention, or a fragment or variant or derivative of said DNA sequence.

[0116] An "isolated" RNA is an RNA which is substantially separated from other mycobacterial components that naturally accompany the sequences of interest, eg., ribosomes, polymerases, and other mycobacterial polynucleotides such as DNA and other chromosomal sequences.

[0117] The above RNA molecule may be introduced directly into a host cell as, for example, a component of a vaccine.

[0118] Alternatively the RNA molecule may be incorporated into an RNA vector prior to administration.

[0119] The polynucleotide sequences (DNA and RNA) of the present invention include a nucleic acid sequence which has been removed from its naturally occurring environment, and recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems.

[0120] The term "recombinant" as used herein intends a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of a polynucleotide with which it is associated in nature; or (2) is linked to a polynucleotide other than that to which it is linked in nature; and (3) does not occur in nature. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, eg., by genetic engineering techniques. Such is usually done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions.

[0121] In embodiments of the invention the polynucleotides may encode a peptide (or fragment, variant, or derivative) which is induced or up-regulated under nutrient-starving conditions. A nucleic acid is said to "encode" a peptide if, in its native state or when manipulated, it can be transcribed and/or translated to produce the peptide (or fragment, variant or derivative thereof). The anti-sense strand of such a nucleic acid is also said to encode the peptide (or fragment, variant, or derivative).

[0122] Also contemplated within the invention are expression vectors comprising the polynucleotide of interest. Expression vectors generally are replicable polynucleotide constructs that encode a peptide operably linked to suitable transcriptional and translational regulatory elements. Examples of regulatory elements usually included in expression vectors are promoters, enhancers, ribosomal binding sites, and transcription and translation initiation and termination sequences. These regulatory elements are operably linked to the sequence to be translated. A nucleic acid sequence is operably linked when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression. Generally, operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. The regulatory elements employed in the expression vectors containing a polynucleotide encoding a virulence factor are functional in the host cell used for expression.

[0123] The polynucleotides of the present invention may be prepared by any means known in the art. For example, large amounts of the polynucleotides may be produced by replication in a suitable host cell. The natural or synthetic DNA fragments coding for a desired fragment will be incorporated into recombinant nucleic acid constructs, typically DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the DNA constructs will be suitable for autonomous replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to and integration within the genome of a cultured insect, mammalian, plant or other eukaryotic cell lines. The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite method or the triester method, and may be performed on commercial automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary

strand using DNA polymerase with an appropriate primer sequence. DNA constructs prepared for introduction into a prokaryotic or eukaryotic host will typically comprise a replication system recognized by the host, including the intended DNA fragment encoding the desired peptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Secretion signals from polypeptides secreted from the host cell of choice may also be included where appropriate, thus allowing the protein to cross and/or lodge in cell membranes, and thus attain its functional topology or be secreted from the cell. Appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may, when appropriate, include those naturally associated with mycobacterial genes. Promoters such as the *trp*, *lac* and phage promoters, tRNA promoters and glycolytic enzyme promoters may be used in prokaryotic hosts. Useful yeast promoters include the promoter regions for metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate dehydrogenase, enzymes responsible for maltose and galactose utilization, and others.

[0124] Appropriate non-native mammalian promoters may include the early and late promoters from SV40 or promoters derived from murine moloney leukemia virus, mouse mammary tumour virus, avian sarcoma viruses, adenovirus II, bovine papilloma virus or polyoma. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. While such expression vectors may replicate autonomously, they may less preferably replicate by being inserted into the genome of the host cell. Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the vector. The presence of this gene ensures the growth of only those host cells which express the inserts. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxic substances, e.g. ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media, e.g. the gene encoding D-alanine racemase for *Bacilli*. The choice of appropriate selectable marker will depend on the host cell. The vectors containing the nucleic acids of interest can be transcribed *in vitro* and the resulting RNA introduced into the host cell (e.g., by injection), or the vectors can be introduced directly into host cells by methods which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an infectious agent, such as a retroviral genome). The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells. Large quantities of the nucleic acids and peptides of the present invention may be prepared by expressing the nucleic acids or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used. Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, amphibian or avian species, may also be useful for production of the proteins of the present invention. Propagation of mammalian cells in culture is *per se* well known. Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although other cell lines may be appropriate, e.g., to provide higher expression, desirable glycosylation patterns. Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. The transformant may be screened or, preferably, selected by any of the means well known in the art, e.g., by resistance to such antibiotics as ampicillin, tetracycline.

[0125] The polynucleotides of the invention may be inserted into the host cell by any means known in the art, including for example, transformation, transduction, and electroporation. As used herein, "recombinant host cells", "host cells", "cells", "cell lines", "cell cultures", and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transfer DNA, and include the progeny of the original cell which has been transformed. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation. "Transformation", as used herein, refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, for example, direct uptake, transduction, f-mating or electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host cell genome.

[0126] In one embodiment, a DNA plasmid or RNA vector may encode a component of the immune system which is specific to an immune response following challenge with a peptide, wherein said peptide is encoded by a mycobacterial gene that is induced or up-regulated during nutrient-starvation, and optionally oxygen starvation.

[0127] An example of such a component is an antibody to the peptide product of the induced or up-regulated gene. Thus, in one embodiment, the nucleic acid sequence (eg. DNA plasmid, or RNA vector) encodes the antibody in question.

[0128] An eighth aspect provides use of the aforementioned aspects of the present invention, namely a peptide or fragment or variant or derivative thereof, an inhibitor, an antibody, an attenuated mycobacterium, an attenuated microbial

carrier, a DNA sequence that is the coding sequence of an induced or up-regulated mycobacterial gene or a fragment or variant or derivative of said coding sequence, a DNA plasmid comprising said DNA sequence, an RNA sequence encoded by said DNA sequence (including DNA fragment, variant, derivative), and/or an RNA vector comprising said RNA sequence, in the manufacture of a medicament for treating or preventing a mycobacterial infection.

[0129] The term "preventing" includes reducing the severity/intensity of, or initiation of, a mycobacterial infection.

[0130] The term "treating" includes post-infection therapy and amelioration of a mycobacterial infection.

[0131] In a related aspect, there is provided a method of treating or preventing a mycobacterial infection, comprising administration of a medicament (namely the aforementioned aspects of the present invention) selected from the group consisting of a peptide or fragment or variant or derivative thereof, an inhibitor, an antibody, an attenuated mycobacterium, an attenuated microbial carrier, a DNA sequence that is the coding sequence of an induced or up-regulated mycobacterial gene or a fragment or variant or derivative of said coding sequence, a DNA plasmid comprising said DNA sequence, an RNA sequence encoded by said DNA sequence, and/or an RNA vector comprising said RNA sequence, to a patient.

[0132] The immunogenicity of the epitopes of the peptides of the invention may be enhanced by preparing them in mammalian or yeast systems fused with or assembled with particle-forming proteins such as, for example, that associated with hepatitis B surface antigen. Vaccines may be prepared from one or more immunogenic peptides of the present invention. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the peptide encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations or formulations suitable for distribution as aerosols. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25%-70%. The peptides may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or with organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like. The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of 5 micrograms to 250 micrograms of antigen per dose, depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgment of the practitioner and may be peculiar to each subject. The vaccine may be given in a single dose schedule, or preferably in a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and/or re-enforce the immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the individual and be dependent upon the judgment of the practitioner. In addition, the vaccine containing the immunogenic mycobacterial antigen(s) may be administered in conjunction with other immunoregulatory agents, for example, immunoglobulins, as well as antibiotics.

[0133] The medicament may be administered by conventional routes, eg. intravenous, intraperitoneal, intranasal routes.

[0134] The outcome of administering antibody-containing compositions may depend on the efficiency of transmission of antibodies to the site of infection. In the case of a mycobacterial respiratory infection (eg. a *M. tuberculosis* infection), this may be facilitated by efficient transmission of antibodies to the lungs.

[0135] In one embodiment the medicament may be administered intranasally (i.n.). This mode of delivery corresponds

to the route of delivery of a *M. tuberculosis* infection and, in the case of antibody delivery, ensures that antibodies are present at the site of infection to combat the bacterium before it becomes intracellular and also during the period when it spreads between cells.

[0136] An intranasal composition may be administered in droplet form having approximate diameters in the range of 100-5000 μm , preferably 500-4000 μm , more preferably 1000-3000 μm . Alternatively, in terms of volume, the droplets would be in the approximate range of 0.001-100 μl , preferably 0.1-50 μl , more preferably 1.0-25 μl .

[0137] Intranasal administration may be achieved by way of applying nasal droplets or via a nasal spray.

[0138] In the case of nasal droplets, the droplets may typically have a diameter of approximately 1000-3000 μm and/or a volume of 1-25 μl .

[0139] In the case of a nasal spray, the droplets may typically have a diameter of approximately 100-1000 μm and/or a volume of 0.001-1 μl .

[0140] It is possible that, following i.n. delivery of antibodies, their passage to the lungs is facilitated by a reverse flow of mucosal secretions, although mucociliary action in the respiratory tract is thought to take particles within the mucus out of the lungs. The relatively long persistence in the lungs' lavage, fast clearance from the bile and lack of transport to the saliva of some antibodies suggest the role of mucosal site specific mechanisms.

[0141] In a different embodiment, the medicament may be delivered in an aerosol formulation. The aerosol formulation may take the form of a powder, suspension or solution.

[0142] The size of aerosol particles is one factor relevant to the delivery capability of an aerosol. Thus, smaller particles may travel further down the respiratory airway towards the alveoli than would larger particles. In one embodiment, the aerosol particles have a diameter distribution to facilitate delivery along the entire length of the bronchi, bronchioles, and alveoli. Alternatively, the particle size distribution may be selected to target a particular section of the respiratory airway, for example the alveoli.

[0143] The aerosol particles may be delivered by way of a nebulizer or nasal spray.

[0144] In the case of aerosol delivery of the medicament, the particles may have diameters in the approximate range of 0.1-50 μm , preferably 1-25 μm , more preferably 1-5 μm .

[0145] The aerosol formulation of the medicament of the present invention may optionally contain a propellant and/or surfactant.

[0146] By controlling the size of the droplets which are to be administered to a patient to within the defined range of the present invention, it is possible to avoid/minimise inadvertent antigen delivery to the alveoli and thus avoid alveoli-associated pathological problems such as inflammation and fibrotic scarring of the lungs.

[0147] I.n. vaccination engages both T and B cell mediated effector mechanisms in nasal and bronchus associated mucosal tissues, which differ from other mucosae-associated lymphoid tissues.

[0148] The protective mechanisms invoked by the intranasal route of administration may include: the activation of T lymphocytes with preferential lung homing; upregulation of co-stimulatory molecules, eg. B7.2; and/or activation of macrophages or secretory IgA antibodies.

[0149] Intranasal delivery of antigens may facilitate a mucosal antibody response is invoked which is favoured by a shift in the T cell response toward the Th2 phenotype which helps antibody production. A mucosal response is characterised by enhanced IgA production, and a Th2 response is characterised by enhanced IL-4 production.

[0150] Intranasal delivery of mycobacterial antigens allows targeting of the antigens to submucosal B cells of the respiratory system. These B cells are the major local IgA-producing cells in mammals and intranasal delivery facilitates a rapid increase in IgA production by these cells against the mycobacterial antigens.

[0151] In one embodiment administration of the medicament comprising a mycobacterial antigen stimulates IgA antibody production, and the IgA antibody binds to the mycobacterial antigen. In another embodiment, a mucosal and/or Th2 immune response is stimulated.

[0152] In another embodiment monoclonal antibodies, in particular, may be used to raise anti-idiotypic antibodies. Anti-idiotypic antibodies are immunoglobulins which carry an "internal image" of the antigen of the infectious agent against which protection is desired. These anti-idiotypic antibodies may also be useful for treatment, vaccination and/or diagnosis of mycobacterial infections.

[0153] According to a ninth aspect of the present invention, the peptides (including fragments, variants, and derivatives thereof) of the present invention and antibodies which bind thereto are useful in immunoassays to detect the presence of antibodies to mycobacteria, or the presence of the virulence associated antigens in biological samples. Design of the immunoassays is subject to a great deal of variation, and many formats are known in the art. The immunoassay may utilize at least one epitope derived from a peptide of the present invention. In one embodiment, the immunoassay uses a combination of such epitopes. These epitopes may be derived from the same or from different bacterial peptides, and may be in separate recombinant or natural peptides, or together in the same recombinant peptides.

[0154] An immunoassay may use, for example, a monoclonal antibody directed towards a virulence associated peptide epitope(s), a combination of monoclonal antibodies directed towards epitopes of one mycobacterial antigen, monoclonal antibodies directed towards epitopes of different mycobacterial antigens, polyclonal antibodies directed towards the

same antigen, or polyclonal antibodies directed towards different antigens. Protocols may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labelled antibody or polypeptide; the labels may be, for example, enzymatic, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labelled and mediated immunoassays, such as ELISA assays. Typically, an immunoassay for an antibody(s) to a peptide, will involve selecting and preparing the test sample suspected of containing the antibodies, such as a biological sample, then incubating it with an antigenic (i.e., epitope-containing) peptide(s) under conditions that allow antigen-antibody complexes to form, and then detecting the formation of such complexes. The immunoassay may be of a standard or competitive type. The peptide is typically bound to a solid support to facilitate separation of the sample from the peptide after incubation. Examples of solid supports that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidene fluoride (known as Immulon), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech Immulon microtiter plates or 60 mm diameter polystyrene beads (Precision Plastic Ball) may be used. The solid support containing the antigenic peptide is typically washed after separating it from the test sample, and prior to detection of bound antibodies. Complexes formed comprising antibody (or, in the case of competitive assays, the amount of competing antibody) are detected by any of a number of known techniques, depending on the format. For example, unlabelled antibodies in the complex may be detected using a conjugate of antixenogeneic Ig complexed with a label, (e.g., an enzyme label). In immunoassays where the peptides are the analyte, the test sample, typically a biological sample, is incubated with antibodies directed against the peptide under conditions that allow the formation of antigen-antibody complexes. It may be desirable to treat the biological sample to release putative bacterial components prior to testing. Various formats can be employed. For example, a "sandwich assay" may be employed, where antibody bound to a solid support is incubated with the test sample; washed; incubated with a second, labelled antibody to the analyte, and the support is washed again. Analyte is detected by determining if the second antibody is bound to the support. In a competitive format, a test sample is usually incubated with antibody and a labelled, competing antigen is also incubated, either sequentially or simultaneously.

Also included as an embodiment of the invention is an immunoassay kit comprised of one or more peptides of the invention, or one or more antibodies to said peptides, and a buffer, packaged in suitable containers.

[0155] As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from an individual, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumours, organs, and also samples of in vitro cell culture constituents (including but not limited to conditioned medium resulting from the growth of cells in cell culture medium, putatively virally infected cells, recombinant cells, and cell components).

[0156] In a related diagnostic assay, the present invention provides nucleic acid probes for detecting a mycobacterial infection.

[0157] Using the polynucleotides of the present invention as a basis, oligomers of approximately 8 nucleotides or more can be prepared, either by excision from recombinant polynucleotides or synthetically, which hybridize with the mycobacterial sequences, and are useful in identification of mycobacteria. The probes are a length which allows the detection of the induced or up-regulated sequences by hybridization. While 6-8 nucleotides may be a workable length, sequences of 10-12 nucleotides are preferred, and at least about 20 nucleotides appears optimal. These probes can be prepared using routine methods, including automated oligonucleotide synthetic methods. For use as probes, complete complementarity is desirable, though it may be unnecessary as the length of the fragment is increased. For use of such probes as diagnostics, the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids contained therein. The resulting nucleic acid from the sample may be subjected to gel electrophoresis or other size separation techniques; alternatively, the nucleic acid sample may be dot blotted without size separation. The probes are usually labeled. Suitable labels, and methods for labeling probes are known in the art, and include, for example, radioactive labels incorporated by nick translation or kinasing, biotin, fluorescent probes, and chemiluminescent probes. The nucleic acids extracted from the sample are then treated with the labeled probe under hybridization conditions of suitable stringencies. The probes may be made completely complementary to the virulence encoding polynucleotide. Therefore, usually high stringency conditions are desirable in order to prevent false positives. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time, and concentration of formamide. It may be desirable to use amplification techniques in hybridization assays. Such techniques are known in the art and include, for example, the polymerase chain reaction (PCR) technique. The probes may be packaged into diagnostic kits. Diagnostic kits include the probe DNA, which may be labelled; alternatively, the probe DNA may be unlabeled and the ingredients for labelling may be included in the kit in separate containers. The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, for example, standards, as well as instructions for conducting the test.

[0158] In a preferred embodiment, a peptide (or fragment or variant or derivative) of the present invention is used in

a diagnostic assay to detect the presence of a T-lymphocyte which T lymphocyte has been previously exposed to an antigenic component of a mycobacterial infection in a patient.

[0159] In more detail, a T-lymphocyte which has been previously exposed to a particular antigen will be activated on subsequent challenge by the same antigen. This activation provides a means for identifying a positive diagnosis of mycobacterial infection. In contrast, the same activation is not achieved by a T-lymphocyte which has not been previously exposed to the particular antigen.

[0160] The above "activation" of a T-lymphocyte is sometimes referred to as a "recall response" and may be measured, for example, by determining the release of interferon (eg. IFN- γ) from the activated T-lymphocyte. Thus, the presence of a mycobacterial infection in a patient may be determined by the release of a minimum concentration of interferon from a T-lymphocyte after a defined time period following *in vitro* challenge of the T-lymphocyte with a peptide (or fragment or variant or derivative) of the present invention.

[0161] In use, a biological sample containing T-lymphocytes is taken from a patient, and then challenged with a peptide (or fragment, variant, or derivative thereof) of the present invention.

[0162] The above T-lymphocyte diagnostic assay may include an antigen presenting cell (APC) expressing at least one major histocompatibility complex (MHC) class II molecule expressed by the patient in question. The APC may be inherently provided in the biological sample, or may be added exogenously. In one embodiment, the T-lymphocyte is a CD4 T-lymphocyte.

Brief mention is now made to the Figures of the present application, in which:-

[0163]

Fig. 1 illustrates the viable counts for *M. tuberculosis* during culture under batch fermentation conditions at a DOT of 50 % air saturation (37 °C); and

Fig. 2 illustrates the concentration of glycerol (as the primary carbon and energy source during culture of *M. tuberculosis* under batch fermentation conditions at a DOT of 50 % air saturation (37 °C).

Fig. 3 illustrates the DOT within the medium of the mycobacterial culture described in Example 18.

Fig. 4 illustrates the viable counts for *M. tuberculosis* during the batch fermentation conditions of Example 18 (ie. carbon-starvation, and oxygen limiting conditions).

Example 1 - culture of mycobacteria

[0164] Two alternative mycobacterial culture methods have been employed to study genes which are up-regulated or induced during mycobacterial latency.

Model 1 - *in vitro* model of mycobacterial persistence under aerobic, nutrient-starved conditions

Materials and Methods

Strain

[0165] Studies were performed with *M. tuberculosis* strain H37Rv (NCTC cat. no. 7416) - a representative strain of *M. tuberculosis*. Stock cultures were grown on Middlebrook 7H10 + OADC for 3 weeks at 37 $\pm$ 2°C.

Culture Medium

[0166] Persistence cultures were established in Middlebrook 7H9 medium supplemented with Middlebrook ADC enrichment, 0.2% Tween 80 and 0.2% glycerol (Table 1). The medium was prepared with high quality water from a Millipore water purification system and filter sterilised by passage through a 0.1 μ m pore size cellulose acetate membrane filter capsule (Sartorius Ltd). The pH was adjusted to 6.6 with concentrated hydrochloric acid.

[0167] Middlebrook 7H10 + OADC agar was used to prepare inoculum cultures, enumerate the number of culturable bacteria in samples, and to assess culture purity.

Culture system

[0168] We previously developed a process for the culture of mycobacteria under controlled and defined conditions - patent application No. PCT/GB00/00760 (WO00/52139). We used this culture system operated as a batch fermenter for the following studies of mycobacterial persistence.

[0169] Culture experiments were performed in a one litre glass vessel operated at a working volume of 750 ml. The culture was agitated by a magnetic bar placed in the culture vessel coupled to a magnetic stirrer positioned beneath the vessel. Culture conditions were continuously monitored by an Anglicon Microlab Fermentation System (Brighton Systems, Newhaven), linked to sensor probes inserted into the culture through sealed ports in the top plate. The oxygen concentration was monitored with a galvanic oxygen electrode (Uniprobe, Cardiff) and was controlled through sparging the culture with a mixture of air and oxygen free-nitrogen. Temperature was monitored by an Anglicon temperature probe, and maintained by a heating pad positioned beneath the culture vessel. Culture pH was measured using an Ingold pH electrode (Mettler-Toledo, Leicester).

Inoculation and culture

[0170] The vessel was filled with 750 ml of sterile culture medium and parameters were allowed to stabilise at 37 °C $\pm$ 2 °C, pH 6.9 $\pm$ 0.3 and a dissolved oxygen tension of approximately 70 % air saturation. A dense inoculum suspension was prepared by resuspending Middlebrook agar cultures, grown at 37 °C $\pm$ 2 °C for 3 weeks, in sterile deionised water. The inoculum was aseptically transferred to the culture vessel, to provide an initial culture turbidity of approximately 0.25 at 540 nm.

[0171] The culture were maintained at 37 °C with an agitation rate of 500 to 750 rpm. The dissolved oxygen tension was maintained between 50 - 70% air saturation with the aid of culture sparging. The initial culture pH was set at approximately 6.7 and was monitored through-out the experiment.

[0172] The culture was maintained for 50 days and samples were removed regularly to monitor growth and survival, nutrient utilisation and gene expression.

Growth and survival

[0173] Bacterial growth and survival was assessed by determining the number of viable cells in the culture system at specific time points. This was achieved by preparing a decimal dilution series of the sample in sterile water and plating 100 μ l aliquots onto Middlebrook 7H10 + OADC plates. The plates were incubated at 37 °C for up to 4 weeks before enumerating the number of colonies formed.

Nutrient utilisation

[0174] Glycerol is the primary carbon and energy source present in Middlebrook 7H9 medium with ADC, 0.2 % Tween and 0.2 % Glycerol. The rate at which glycerol was utilised was determined using the Glycerol Determination Kit Cat. No. 1448 270 Boehringer Mannheim.

Microarray experiments

[0175] RNA was extracted from culture samples collected at different time points during the experiment. A fluorescently-labelled cDNA was then transcribed from each sample of RNA. The cDNA was labelled by the incorporation of either Cy3 or Cy5 labelled dCTP (Dyes are supplied by Amersham Pharmacia Biotech).

[0176] Whole *M. tuberculosis* genome arrays were prepared from *M. tuberculosis* genomic DNA using ORF-specific primers. PCR products corresponding to each ORF were spotted in a grid onto a standard glass microscope slide using a BioRobotics microgrid robot (MWG Biotech) at a resolution of >4000 spots/cm².

[0177] In each microarray experiment a whole genome array was hybridised with labelled cDNA from one culture sample (Test sample). Each array was also hybridised with control DNA incorporating a different Cy dye and prepared from DNA extracted from *M. tuberculosis* strain H37Rv (control sample).

[0178] Each array was scanned at two different wavelengths corresponding to the excitation maxima of each dye and the intensity of the emitted light was recorded. The ratio of the intensity values for the test and control samples was determined for each array.

[0179] The slides were scanned using an Affymetrix 428 scanner. The raw data was initially analysed by ImaGene software. The scanned images were then transferred to another software package known as GeneSpring to analyse the expression of each gene.

Results

[0180] After inoculation the culture entered exponential growth and continued to grow exponentially until 10 days after inoculation (see Fig. 1). Cessation of exponential growth coincided with depletion of the primary carbon and energy source - glycerol (see Fig. 2). As the culture entered stationary phase, viability started to decline and continued to decline steadily over the duration of the study. After 40 days in stationary phase, approximately 1% of the culture was still culturable on Middlebrook agar.

[0181] The gene expression profiles for samples collect at day 5 and day 50 were compared. Three arrays were prepared for each sample and the ratio of the intensity values for the test and control samples was determined for each array.

[0182] Two different approaches were used to analyse the data:-

1. The ratio values for the 3 arrays prepared for each sample were averaged and compared. Genes which produced intensity ratios that were 3-fold higher on day 50 than on day 5 were selected.

2. Data from each array was treated as a separate data set and self-organising maps were used to select all the genes that were consistently up-regulated in all 3 arrays at day 50 relative to day 5.

[0183] The two data sets were then compared and those genes that were at least 1.5-fold, preferably at least 3-fold up-regulated at day 50, relative to exponential growth at day 5, and which were consistently up-regulated in all 3 arrays (experiments) were selected. The identified sequences (protein, followed by nucleic acid) are presented in Table 2.

Model 2 - *in vitro* model of mycobacterial persistence under low oxygen, and nutrient-starved conditions

[0184] A second model which simulated low-oxygen availability and nutrient depletion has also been developed. This model was established as outlined for Model 1 above, but with the following modifications.

[0185] After inoculation, the dissolved oxygen tension (DOT) of the culture was maintained at approximately 40% air saturation at 37 °C until the culture had entered early exponential growth. The DOT was then lowered in increments down to 1% air saturation over a six day period. The culture was then maintained at a DOT of 0 - 5% until 50 days after inoculation. Samples were collected for analysis, and the identified sequences (protein, followed by nucleic acid) are presented in Table 2.

Table 1 - liquid medium formulation for persistence cultures - Middlebrook 7H9 medium supplemented with ADC, 0.2% Tween 80 and 0.2% Glycerol

Composition per litre	
Na ₂ HPO ₄	2.5 g
KH ₂ PO ₄	1.0 g
Monosodium glutamate	0.5 g
(NH ₄) ₂ SO ₄	0.5 g
Sodium citrate	0.1 g
MgSO ₄ ·7H ₂ O	0.05 g
Ferric ammonium citrate	0.04 g
CuSO ₄ ·5H ₂ O	1.0 mg
Pyridoxine	1.0 mg
ZnSO ₄ ·7H ₂ O	1.0 mg
Biotin	0.5 mg
CaCl ₂ ·2H ₂ O	0.5 mg
Middlebrook ADC enrichment	100 ml
Glycerol	2.0 ml
Tween 80	2.0 ml
Middlebrook ADC enrichment - per	100 ml
Bovine serum albumin	5.0 g

(continued)

Composition per litre

Glucose	2.0 g
Catalase	3.0 mg

[0186] Example 2 - RNA extraction from *M. tuberculosis* for microarray analysis**Materials and Methods**

[0187] Trizol (Life Technologies)- formulation of phenol and guanidine thiocyanate.

[0188] GTC lysis solution containing: 5 M guanidine thiocyanate, 0.5 % N-lauryl sarcosine, 25 mM tri-sodium citrate, 0.1 M 2-mercaptoethanol, and 0.5 % Tween 80.

Chloroform
Isopropanol
3M sodium acetate
70 % Ethanol
microfuge
ribolyser

Sterile plasticware-Falcon tubes, screw capped eppendorfs, gilson tips -all RNase free
Glassware - baked at 160 °C for at least 16 hours

Method

[0189] Steps performed at Containment level 3; within a Class III microbiological safety cabinet.

[0190] Remove 10 or 20 ml of culture (10⁹/ml) and immediately add this to 4 volumes of GTC lysis buffer in a plastic specimen pot. Seal the pot tightly.

[0191] Incubate the cells in GTC lysis buffer for 1 hour at room temperature. Surface decontaminate the plastic pot with 5 % Hycolin for 5 minutes. Transfer the sample to the pass box and place it into a plastic carry tin with a sealable lid. Close the container securely and transport it to a non-toxic cabinet CL3 cabinet.

[0192] Equally distribute the lysis mixture between Falcon tubes. Place these tubes into centrifuge buckets and seal the buckets tightly. Surface-decontaminate the buckets for 5 minutes with 5 % Hycolin. Then transfer them to the centrifuge (Baird and Tatlock Mark IV refrigerated bench-top centrifuge). Spin the tubes at 3,000 rpm for 30 minutes.

[0193] Return the unopened buckets to the cabinet. Remove the centrifuge tubes and pour the supernatant into a waste bottle for GTC lysis buffer.

[0194] Resuspend each pellet in 1 ml of Trizol (formulation of phenol and GTC cat no. 15596-026). The manufacturers guidelines recommend lysing cells by repetitive pipetting. Although this action alone will not lyse *M. tuberculosis*, it is important to completely resuspend the pellet in Trizol.

[0195] Transfer 1 ml of cells into a FastRNA tube and ribolyse it at power setting 6.5 for 45 seconds.

[0196] Leave the tube to incubate at room temperature for 5 minutes.

[0197] Remove the aqueous layer from the tube and add this to 200 µl of chloroform in a screw-capped eppendorf tube. Shake each tube vigorously for about 15 seconds. Incubate for 2-3 minutes at room temperature.

[0198] Spin the tube at 13,000 rpm for 15 minutes. Following centrifugation, the liquid separates into red phenol/chloroform phase, an interface, and a clear aqueous phase.

[0199] Carefully remove the aqueous phase and transfer it to a fresh eppendorf tube containing 500 µl of chloroform/isoamyl alcohol (24:1). Spin the tubes at 13,000 rpm for 15 minutes.

[0200] Transfer the aqueous phase to an eppendorf tube containing 50 µl of sodium acetate and 500 µl of isopropanol.

[0201] Surface decontaminate the eppendorf tube with 5% Hycolin for 5 minutes. Remove the tube from the CL3 laboratory and continue with the procedure in laboratory 157.

Steps performed at Containment level 2:

[0202] Precipitate the RNA at -70 °C for at least 30 minutes-can do this step overnight.

[0203] Spin the precipitated RNA down at 13,000 rpm for 10 minutes. Remove the supernatant and wash the pellet in 70 % ethanol. Repeat centrifugation.

[0204] Remove the 70 % ethanol and air-dry the pellet. Dissolve the pellet in RNase free water.

[0205] Freeze the RNA at -70 °C to store it.

Example 3 - isolation of genomic DNA from *Mycobacterium tuberculosis* grown in chemostat culture. DNA then used to generate Cy3 or Cy5 labelled DNA for use as a control in microarray experiments.

Materials and Methods

[0206]

Beads 0.5 mm in diameter
Bead beater
Bench top centrifuge
Platform rocker
Heat block
Falcon 50 ml centrifuge tubes
Sorvall RC-5C centrifuge
250 ml polypropylene centrifuge pots.
Screw capped eppendorf tubes
Pipettes 1 ml, 200 µl, 10 ml, 5 ml
Breaking buffer
50 mM Tris HCl pH 8.0
10 mM EDTA
100 mM NaCl

Procedure

Mechanical disruption of *M. tuberculosis* cells

[0207]

- * 150 ml of chemostat cells (O.D of 2.5 at 540 nm) are spun down at 15,000 rpm for 15 minutes in 250 ml polypropylene pots using centrifuge Sorvall RC-5C.
- * The supernatant is discarded.
- * Cells are re-suspended in 5 ml of breaking buffer in a 50 ml Falcon tube and centrifuged at 15,000 rpm for a further 15 minutes.
- * The supernatant is removed and additional breaking buffer is added at a volume of 5 ml. Beads are used to disrupt the cells. These are used at a quantity of 1 ml of beads for 1 ml of cells. Place the sample into the appropriate sized chamber. Place in the bead beater and secure the outer unit (containing ice) and process at the desired speed for 30 seconds.
- * Allow the beads to settle for 10 minutes and transfer cell lysate to a 50 ml Falcon centrifuge tube
- * Wash beads with 2-5 ml of breaking buffer by pipetting washing buffer up and down over the beads.
- * Add this washing solution to the lysate in the falcon tube

Removal of proteins and cellular components

[0208]

- * Add 0.1 volumes of 10% SDS and 0.01 volumes proteinase K.
- * Mix by inversion and heat at 55 °C in a heat block for 2-3 hours
- * The resulting mix should be homogenous and viscous. Additional SDS may be added to assist here to bring the concentration up to 0.2 %
- * Add an equal volume of phenol/chloroform/Isoamyl alcohol in the ratio: 25/24/1.
- * Gently mix on a platform rocker until homogenous
- * Spin down at 3,000 rpm for 20 minutes
- * Remove the aqueous phase and place in a fresh tube
- * Extract the aqueous phase with an equal volume of chloroform to remove traces of cell debris and phenol. Chloroform extractions may need to be repeated to remove all the debris.
- * Precipitate the DNA with 0.3 M sodium acetate and an equal volume of isopropanol.

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- * Spool as much DNA as you can with a glass rod
- * Wash the spooled DNA in 70 % ethanol followed by 100 % ethanol
- * Leave to air dry
- * Dissolve the DNA in sterile deionised water (500 μ l)
- * Allow DNA to dissolve at 4 °C for approximately 16 hours.
- * Add RNase 1 (500U) to the dissolved DNA
- * Incubate for 1 hour at 37°C.
- * Re-extract with an equal volume of phenol/chloroform followed by a chloroform extraction and precipitate as before
- * Spin down the DNA at 13,000 rpm
- * Remove the supernatant and wash the pellet in 70% ethanol
- * Air dry
- * Dissolve in 200-500 μ l of sterile water.

Example 4 - preparation of Cy3 or Cy5 labelled DNA from DNA

a) Prepare one Cy3 or one Cy5 labelled DNA sample per microarray slide.

[0209] Each sample:

DNA	2-5 μ g
Random primers (3 μ g/ μ l)	1 μ l
H ₂ O	to 41.5 μ l

[0210] Heat at 95°C for 5 min, snap cool on ice and briefly centrifuge.

Add to each:

[0211]

10 x REact 2 buffer	5 μ l
dNTPs (5mM dA/G/TTP, 2 mM dCTP)	1 μ l
Cy3 OR Cy5 dCTP	1.5 μ l
Klenow (5U/ μ l)	1 μ l
Incubate at 37°C in dark for 90 min.	

b) Prehybridise slide

[0212] Mix the prehybridisation solution in a Coplin jar and incubate at 65 °C during the labelling reaction to equilibrate.

Prehybridisation: 20x SSC	8.75 ml (3.5 x SSC)
20% SDS	250 μ l (0.1% SDS)
BSA (100 mg/ml)	5 ml (10 mg/ml)
H ₂ O	to 50 ml

[0213] Incubate the microarray slide in the pre-heated prehybridisation solution at 65 °C for 20 min. Rinse slide thoroughly in 400 ml H₂O for 1 min followed by rinse in 400 ml propan-2-ol for 1 min and centrifuge slide in 50 ml centrifuge tube at 1,500 rpm for 5 min to dry. Store slide in dark, dust-free box until hybridisation (<1h).

c) Purify Cy3/Cy5 labelled DNA - Qiagen MinElute Purification

[0214]

- * Combine Cy3 and Cy5 labelled DNA samples in single tube and add 500 μ l Buffer PB.
- * Apply to MinElute column in collection tube and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.

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- * Add 500 µl Buffer PE to MinElute column and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Add 250 µl Buffer PE to MinElute column and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Centrifuge at 13,000 rpm for an additional 1 min to remove residual ethanol.
- * Place the MinElute column into a fresh 1.5 ml tube.
- * Add 10.5 µl H₂O to the centre of the membrane and allow to stand for 1 min.
- * Centrifuge at 13,000 rpm for 1 min.

Example 5 - preparation of Cy3 or Cy5 label cDNA from RNA

a) Prepare one Cy3 and one Cy5 labelled cDNA sample per microarray slide

[0215] Each sample:

RNA	2-10 µg
Random primers (3(µg/µl)	1 µl
H ₂ O	to 11 µl

[0216] Heat at 95°C for 5min, snap cool on ice and briefly centrifuge.

Add to each: 5lFirst Strand Buffer	5 µl
DTT (100 mM)	2.5 µl
dNTPs (5 mM dA/G/TTP, 2 mM dCTP)	2.3 µl
Cy3 OR Cy5 dCTP	1.7 µl
Superscript II (200 U/µl)	2.5 µl

[0217] Incubate at 25 °C in dark for 10 min followed by 42°C in dark for 90 min.

b) Prehybridise slide

[0218] Mix the prehybridisation solution in a Coplin jar and incubate at 65 °C during the labelling reaction to equilibrate.

[0219] Prehybridisation:

20 x SSC	8.75 ml (3.5 x SSC)
20% SDS	250 µl (0.1 % SDS)
BSA (100 mg/ml)	5 ml (10 mg/ml)
H ₂ O	to 50 ml

[0220] Incubate the microarray slide in the pre-heated prehybridisation solution at 65 °C for 20 min. Rinse slide thoroughly in 400 ml H₂O for 1 min followed by rinse in 400 ml propan-2-ol for 1 min and centrifuge slide in 50 ml centrifuge tube at 1500 rpm for 5 min to dry. Store slide in dark, dust-free box until hybridisation (<1 h).

c) Purify Cy3/Cy5 labelled cDNA - Qiagen MinElute Purification

[0221]

- * Combine Cy3 and Cy5 labelled DNA samples in single tube and add 250 µl Buffer PB.
- * Apply to MinElute column in collection tube and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Add 500 µl Buffer PE to MinElute column and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Add 250 µl Buffer PE to MinElute column and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Centrifuge at 13,000 rpm for an additional 1 min to remove residual ethanol.

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- * Place the MinElute column into a fresh 1.5 ml tube.
- * Add 10.5 µl H₂O to the centre of the membrane and allow to stand for 1 min.
- * Centrifuge at 13,000 rpm for 1 min.

5 Example 6 - hybridise slide with Cy3/Cy5 labelled cDNA

[0222] Place the prehybridise microarray slide in the hybridisation cassette and add two 15 ml aliquots of H₂O to the wells in the cassette. Mix resuspended Cy3/Cy5 labelled cDNA sample with hybridisation solution.

10	Hybridisation: Cy3/Cy5 labelled cDNA sample	10.5 ml
	20xSSC	3.2 ml (4 x SSC)
	2% SDS	2.3 ml (0.3% SDS)

15 [0223] Heat hybridisation solution at 95°C for 2 min. Do not snap cool on ice but allow to cool slightly and briefly centrifuge. Pipette the hybridisation solution onto the slide at the edge of the arrayed area avoiding bubble formation. Using forceps carefully drag the edge of a cover slip along the surface of the slide towards the arrayed area and into the hybridisation solution at the edge of the array. Carefully lower the cover slip down over the array avoiding any additional movement once in place. Seal the hybridisation cassette and submerge in a water bath at 60 °C for 16-20 h.

20 [0224] Wash slide.

[0225] Remove microarray slide from hybridisation cassette and initially wash slide carefully in staining trough of Wash A to remove cover slip. Once cover slip is displaced place slide(s) in slide rack and continue agitating in Wash A for a further 2 min.

25	Wash A:	20xSSC	20 ml (1 x SSC)
		20% SDS	1 ml(0.05% SDS)
		H ₂ O	.to 400 ml

30 [0226] Transfer slide(s) to a clean slide rack and agitate in first trough of Wash B for 2 min. Wash in second trough of Wash B with agitation for 2 min.

Wash B	(x2):20xSSC	1.2 ml (0.06 x SSC)
	H ₂ O	to 400 ml

35 [0227] Place slide into a 50 ml centrifuge tube and centrifuge at 1500 rpm for 5 mins to dry slide, and then scan fluorescence using a ScanArray 3000 dual-laser confocal scanner and analyse data.

Example 7 - preparation of the arrays

40 [0228] PCR-amplified products are generated from *M. tuberculosis* genomic DNA using ORF-specific primers. Each gene of the genome is represented. These are spotted in a grid onto a standard glass microscope slide using a BioRobotics microgrid robot (MWG Biotech) at a resolution of >4000 spots/cm².

45 Example 8 - scanning and analysis of data

[0229] The slides were scanned using an Affymetrix 428 scanner.

50 [0230] Dual fluorescence is used, allowing simultaneous detection of two cDNA samples. The output of the arrays is read using a confocal laser scanner (Affymetrix 428 scanner from MWG Biotech). More detailed information can be found web site www.sghms.ac.uk/depts/medmicro/bugs; Mujumdar, R.B. (1993) Bioconjugate Chemistry, 4(2), pp. 105-111; Yu, H. (1994) Nucl. Acids Res. 22, pp.3226-3232; and Zhu, Z. (1994) Nucl. Acids Res. 22, pp. 3418-3422.

[0231] The raw data were initially analysed in software known as ImaGene, which was supplied with the scanner. The scanned images were then transferred to another software package known as GeneSpring. This is a very powerful tool, which draws information from many databases allowing the complete analysis of the expression of each gene.

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Example 9 - delete one or more of the genes from *M. tuberculosis* in order to attenuate its virulence while retaining immunogenicity

[0232] One or more genes that are identified may be disrupted using allelic exchange. In brief, the gene of interest is cloned with 1-2 kb of flanking DNA either side and is inactivated by deletion of part of the coding region and insertion of an antibiotic resistance marker, such as hygromycin.

[0233] The manipulated fragment is then transferred to a suitable suicide vector e.g. pPR23 and is transformed into the wild-type parent strain of *M. tuberculosis*. Mutants are recovered by selecting for antibiotic resistant strains. Genotypic analysis (Southern Blotting with a fragment specific to the gene of interest) is performed on the selected strains to confirm that the gene has been disrupted.

[0234] The mutant strain is then studied to determine the effect of the gene disruption on the phenotype. In order to use it as a vaccine candidate it would be necessary to demonstrate attenuated virulence. This can be done using either a guinea pig or mouse model of infection. Animals are infected with the mutant strain and the progression of disease is monitored by determining the bacterial load in different organs, in particular the lung and spleen, at specific time points post infection, typically up to 16 weeks.

[0235] Comparison is made to animals infected with the wild-type strain which should have a significantly higher bacterial load in the different organs. Long-term survival studies and histopathology can also be used to assess virulence and pathogenicity.

[0236] Once attenuated virulence has been established, protection and immunogenicity studies can be performed to assess the potential of the strain as a vaccine. Suitable references for allelic exchange and preparation of TB mutants are McKinney et al., 2000 and Pelicic et al., 1997, [1, 2].

Example 10 - select one or more of the genes identifiable by the present invention, which encode proteins that are immunogenic, and put them into BCG or an attenuated strain of *M. tuberculosis* to enhance its overall immunogenicity

[0237] The gene of interest is amplified from the *M. tuberculosis* genome by PCR. The amplified product is purified and cloned into a plasmid (pMV306) that integrates site specifically into the mycobacterial genome at the attachment site (attB) for mycobacteriophage L5 [3].

[0238] BCG is transformed with the plasmid by electroporation, which involves damaging the cell envelope with high voltage electrical pulses, resulting in uptake of the DNA. The plasmid integrates into the BCG chromosome at the attB site generating stable recombinants. Recombinants are selected and are checked by PCR or Southern blotting to ensure that the gene has been integrated. The recombinant strain is then used for protection studies.

Example 11 - Use of recombinant carriers such as attenuated salmonella and the Vaccinia virus to express and present TB genes.

[0239] One of the best examples of this type of approach is the use of Modified Vaccinia virus Ankara (MVA) [4]. The gene of interest is cloned into a vaccinia virus shuttle vector, e.g. pSC11. Baby Hamster Kidney (BHK) cells are then infected with wild-type MVA and are transfected with the recombinant shuttle vector. Recombinant virus is then selected using a suitable selection marker and viral plaques, selected and purified.

[0240] Recombinant virus is normally delivered as part of a prime-boost regime where animals are vaccinated initially with a DNA vaccine encoding the TB genes of interest under the control of a constitutive promoter. The immune response is boosted by administering recombinant MVA carrying the genes of interest to the animals at least 2 weeks later.

Example 12 - Sub-unit vaccines containing a single peptide/protein or a combination of proteins

[0241] To prepare sub-unit vaccines with one or more peptides or proteins it is first of all necessary to obtain a supply of protein or peptide to prepare the vaccine. Up to now, this has mainly been achieved in mycobacterial studies by purifying proteins of interest from TB culture. However, it is becoming more common to clone the gene of interest and produce a recombinant protein.

[0242] The coding sequence for the gene of interest is amplified by PCR with restriction sites inserted at the N terminus and C terminus to permit cloning in-frame into a protein expression vector such as pET-15b. The gene is inserted behind an inducible promoter such as lacZ. The vector is then transformed into *E. coli* which is grown in culture. The recombinant protein is over-expressed and is purified.

[0243] One of the common purification methods is to produce a recombinant protein with an N-terminal His-tag. The protein can then be purified on the basis of the affinity of the His-tag for metal ions on a Ni-NTA column after which the His-tag is cleaved. The purified protein is then administered to animals in a suitable adjuvant [5].

Example 13 - Plasmid DNA vaccines carrying one or more of the identified genes

[0244] DNA encoding a specific gene is amplified by PCR, purified and inserted into specialised vectors developed for vaccine development, such as pVAX1. These vectors contain promoter sequences, which direct strong expression of the introduced DNA (encoding candidate antigens) in eukaryotic cells (e.g. CMV or SV40 promoters), and polyadenylation signals (e.g. SV40 or bovine growth hormone) to stabilise the mRNA transcript.

[0245] The vector is transformed into *E. coli* and transformants are selected using a marker, such as kanamycin resistance, encoded by the plasmid. The plasmid is then recovered from transformed colonies and is sequenced to check that the gene of interest is present and encoded properly without PCR generated mutations.

[0246] Large quantities of the plasmid is then produced in *E. coli* and the plasmid is recovered and purified using commercially available kits (e.g. Qiagen Endofree-plasmid preparation). The vaccine is then administered to animals for example by intramuscular injection in the presence or absence of an adjuvant.

Example 14 - Preparation of DNA expression vectors

[0247] DNA vaccines consist of a nucleic acid sequence of the present invention cloned into a bacterial plasmid. The plasmid vector pVAX1 is commonly used in the preparation of DNA vaccines. The vector is designed to facilitate high copy number replication in *E. coli* and high level transient expression of the peptide of interest in most mammalian cells (for details see manufacturers protocol for pVAX1 (catalog No. V260-20 www.invitrogen.com) .

[0248] The vector contains the following elements:-

- * Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a variety of mammalian cells
- * T7 promoter/priming site to allow *in vitro* transcription in the sense orientation and sequencing through the insert
- * Bovine growth hormone (BGH) polyadenylation signal for efficient transcription termination and polyadenylation of mRNA
- * Kanamycin resistance gene for selection in *E. coli*
- * A multiple cloning site
- * pUC origin for high-copy number replication and growth in *E. coli*
- * BGH reverse priming site to permit sequencing through the insert

[0249] Vectors may be prepared by means of standard recombinant techniques which are known in the art, for example Sambrook et al., (1989). Key stages in preparing the vaccine are as follows:

- * The gene of interest is ligated into pVAX1 via one of the multiple cloning sites
- * The ligation mixture is then transformed into a competent *E. coli* strain (e.g. TOP10) and LB plates containing 50µg/ml kanamycin are used to select transformants.
- * Clones are selected and may be sequenced to confirm the presence and orientation of the gene of interest.
- * Once the presence of the gene has been verified, the vector can be used to transfect a mammalian cell line to check for protein expression. Methods for transfection are known in the art and include, for example, electroporation, calcium phosphate, and lipofection.
- * Once peptide expression has been confirmed, large quantities of the vector can be produced and purified from the appropriate cell host, e.g. *E. coli*.

[0250] pVAX1 does not integrate into the host chromosome. All non-essential sequences have been removed to minimise the possibility of integration. When constructing a specific vector, a leader sequence may be included to direct secretion of the encoded protein when expressed inside the eukaryotic cell.

[0251] Other examples of vectors that have been used are V1Jns.tPA and pCMV4 (Lefevre *et al.*, 2000 and Vordermeier *et al.*, 2000).

[0252] Expression vectors may be used that integrate into the genome of the host, however, it is more common and more preferable to use a vector that does not integrate. The example provided, pVAX1, does not integrate. Integration would lead to the generation of a genetically modified host which raises other issues.

Example 15 - RNA vaccine

[0253] As discussed on page 15 of US patent US5,783,386, one approach is to introduce RNA directly into the host.

[0254] Thus, the vector construct (Example 10) may be used to generate RNA *in vitro* and the purified RNA then injected into the host. The RNA would then serve as a template for translation in the host cell. Integration would not occur.

[0255] Another option is to use an infectious agent such as the retroviral genome carrying RNA corresponding to the

gene of interest. Here you will get integration into the host genome

[0256] Another option is the use of RNA replicon vaccines which can be derived from virus vectors such as Sindbis virus or Semliki Forest virus. These vaccines are self-replicating and self-limiting and may be administered as either RNA or DNA which is then transcribed into RNA replicons *in vivo*. The vector eventually causes lysis of the transfected cells thereby reducing concerns about integration into the host genome. Protocols for RNA vaccine construction are detailed in Cheng *et al.*, (2001).

Example 16 - Diagnostic assays based on assessing T cell responses

[0257] For a diagnostic assay based on assessing T cell responses it would be sufficient to obtain a sample of blood from the patient. Mononuclear cells (monocytes, T and B lymphocytes) can be separated from the blood using density gradients such as Ficoll gradients.

[0258] Both monocytes and B-lymphocytes are both able to present antigen, although less efficiently than professional antigen presenting cells (APCs) such as dendritic cells. The latter are more localised in lymphoid tissue.

[0259] The simplest approach would be to add antigen to the separated mononuclear cells and incubate for a week and then assess the amount of proliferation. If the individual had been exposed to the antigen previously through infection, then T-cell clones specific to the antigen should be more prevalent in the sample and should respond.

[0260] It is also possible to separate the different cellular populations should it be desired to control the ratio of T cells to APC's.

[0261] Another variation of this type of assay is to measure cytokine production by the responding lymphocytes as a measure of response. The ELISPOT assay described below in Example 17 is a suitable example of this variation.

Example 17 - detection of latent mycobacteria

[0262] A major problem for the control of tuberculosis is the presence of a large reservoir of asymptomatic individuals infected with tubercle bacilli. Dormant bacilli are more resistant to front-line drugs.

The presence of latent mycobacteria-associated antigen may be detected indirectly either by detecting antigen specific antibody or T-cells in blood samples.

[0263] The following method is based on the method described in Lalvani *et al.* (2001) in which a secreted antigen, ESAT-6, was identified as being expressed by members of the *M. tuberculosis* complex but is absent from *M. Bovis* BCG vaccine strains and most environmental mycobacteria. 60 - 80% of patients also have a strong cellular immune response to ESAT-6. An *ex-vivo* ELISPOT assay was used to detect ESAT-6 specific T cells.

[0264] As applied to the present invention:

A 96 well plate is coated with cytokine (e.g. interferon- γ , IL-2) -specific antibody. Peripheral blood monocytes are then isolated from patient whole blood and are applied to the wells.

[0265] Antigen (ie. one of the peptides, fragments, derivatives or variants of the present invention) is added to stimulate specific T cells that may be present and the plates are incubated for 24h. The antigen stimulates cytokine production which then binds to the specific antibody.

[0266] The plates are washed leaving a footprint where antigen-specific T cells were present.

[0267] A second antibody coupled with a suitable detection system, e.g. enzyme, is then added and the number of spots are enumerated after the appropriate substrate has been added.

[0268] The number of spots, each corresponding to a single antigen-specific T cell, is related to the total number of cells originally added.

[0269] The above Example also describes use of an antigen that may be used to distinguish TB infected individuals from BCG vaccinated individuals. This could be used in a more discriminative diagnostic assay.

Example 18 - *In vitro* model for mycobacterial persistence under the joint conditions of carbon-starvation and oxygen-limitation (a variation on Examples 1-7)

Materials and Methods

Strain

[0270] Studies were performed with *M. tuberculosis* strain H37Rv (NCTC cat. No. 7416) - a representative strain of *M. tuberculosis*. Stock cultures were grown on Middlebrook 7H10 + OADC for 3 weeks at 37 ± 2 °C.

Culture Medium

[0271] Persistence cultures were established in Middlebrook 7H9 medium supplemented with Middlebrook ADC enrichment, 0.2 % Tween 80 and 0.2 % glycerol (see below). The medium was prepared with high quality water from a Millipore water purification system and filter sterilised by passage through a 0.1 µm pore size cellulose acetate membrane filter capsule (Sartorius Ltd). The pH was adjusted to 6.6 with concentrated hydrochloric acid.

[0272] Middlebrook 7H10 + OADC agar was used to prepare inoculum cultures, enumerate the number of culturable bacteria in samples, and to assess culture purity.

Culture system

[0273] The culture system described in WO00/52139, operated as a batch fermenter, was employed for this Example.

[0274] Culture experiments were performed in a one litre glass vessel operated at a working volume of 750 ml. The culture was agitated by a magnetic bar placed in the culture vessel coupled to a magnetic stirrer positioned beneath the vessel. Culture conditions were continuously monitored by an Anglicon Microlab Fermentation System (Brighton Systems, Newhaven), linked to sensor probes inserted into the culture through sealed ports in the top plate. The oxygen concentration was monitored with a galvanic oxygen electrode (Uniprobe, Cardiff) and was controlled through sparging the culture with a mixture of air and oxygen free-nitrogen. Temperature was monitored by an Anglicon temperature probe, and maintained by a heating pad positioned beneath the culture vessel. Culture pH was measured using an Ingold pH electrode (Mettler-Toledo, Leicester).

Inoculation and culture

[0275] The vessel was filled with 750 ml of sterile culture medium and parameters were allowed to stabilise at 37 °C ± 2°C, pH 6.9 ± 0.3 and a dissolved oxygen tension of approximately 70 % air saturation. A dense inoculum suspension was prepared by resuspending Middlebrook agar cultures, grown at 37 ± 2 °C for 3 week, in sterile deionised water. The inoculum was aseptically transferred to the culture vessel, to provide an initial culture turbidity of approximately 0.25 at 540 nm. The culture was maintained at 37°C with an agitation rate of 500 to 750 rpm.

After inoculation, the dissolved oxygen tension (DOT) of the culture was maintained at approximately 40% air saturation at 37 °C until the culture had entered early exponential growth. The DOT was then lowered in increments down to 1 % air saturation over a six day period (Fig. 3). The culture was then maintained at a DOT of 0 - 5 % until 50 days after inoculation and samples were removed regularly to monitor growth and survival, nutrient utilisation and gene expression.

Growth and survival

[0276] Bacterial growth and survival was assessed by determining the number of viable cells in the culture system at specific time points. This was achieved by preparing a decimal dilution series of the sample in sterile water and plating 100(µl aliquots onto Middlebrook 7H10 + OADC plates. The plates were incubated at 37°C for up to 4 weeks before enumerating the number of colonies formed.

Nutrient utilisation

[0277] Glycerol is the primary carbon and energy source present in Middlebrook 7H9 medium with ADC, 0.2 % Tween and Glycerol. The rate at which glycerol was utilised was determined using the Glycerol Determination Kit Cat. No. 148 270 Boehringer Mannheim.

Microarray experiments

[0278] RNA was extracted from culture samples collected at different time points during the experiment. A fluorescently-labelled cDNA was then transcribed from each sample of RNA. The cDNA was labelled by the incorporation of either Cy3 or Cy5 labelled dCTP (Dyes are supplied by Amersham Pharmacia Biotech).

[0279] Whole *M. tuberculosis* genome arrays were prepared from *M. tuberculosis* genomic DNA using ORF-specific primers. PCR products corresponding to each ORF were spotted in a grid onto a standard glass microscope slide using a BioRobotics microgrid robot (MWG Biotech) at a resolution of >4000 spots/cm². Arrays were supplied by Dr P Butcher, St George's Hospital Medical School London.

[0280] In each microarray experiment a whole genome array was hybridised with labelled cDNA from one culture sample (Test sample). Each array was also hybridised with control DNA incorporating a different Cy dye and prepared from DNA extracted from *M. tuberculosis* strain H37Rv (control sample). Each array was scanned, using an Affymetrix

428 scanner, at two different wavelengths corresponding to the excitation maxima of each dye and the intensity of the emitted light was recorded. The raw data was processed by ImaGene software before performing comparative analysis using GeneSpring.

Results

[0281] Analysis of viable count data indicated that the culture grew exponentially until 10 to 12 days post infection (Fig. 4). As the culture entered stationary phase, viability started to decline and continued to decline steadily over the duration of the study. After 40 days in stationary phase, approximately 0.1 % of the culture was still culturable on Middlebrook agar. The rate of glycerol utilisation was slower than observed in the culture established under aerobic conditions, indicating that the metabolic activity of the low-oxygen culture was restricted by limited oxygen availability. Nevertheless, the principal carbon and energy source was depleted within 15 days after inoculation (Fig. 2).

[0282] Samples were collected for microarray analysis as outlined. The gene expression profiles for samples collected at day 5 and 50 were compared. Three arrays were prepared for each sample and the test data was normalised against the control data on each chip. The normalised data for each set of arrays was then averaged and the two data sets were compared. Those genes that were expressed at least 1.5-fold, preferably at least 5-fold higher at day 50 relative to day 5 were selected. The gene list was then added to those genes identified under "nutrient-starving" conditions, and the complete set listed in Table 2.

[0283] Liquid medium formulation for persistence cultures - Middlebrook 7H9 medium supplemented with ADC, 0.2 % Tween 80 and 0.2 % Glycerol

Composition per litre:

[0284]

Na ₂ HPO ₄	2.5 g
KH ₂ PO ₄	1.0 g
Monosodium glutamate	0.5 g
(NH ₄) ₂ SO ₄	0.5 g
Sodium citrate	0.1 g
MgSO ₄ ·7H ₂ O	0.05 g
Ferric ammonium citrate	0.04 g
CuSO ₄ ·5H ₂ O	1.0 mg
Pyridoxine	1.0 mg
ZnSO ₄ ·7H ₂ O	1.0 mg
Biotin	0.5 mg
CaCl ₂ ·2H ₂ O	0.5 mg

Middlebrook ADC enrichment	100 ml
Glycerol	2.0 ml
Tween 80	2.0 ml

Middlebrook ADC enrichment - per 100 ml

Bovine serum albumin	5.0 g
Glucose	2.0 g
Catalase	3.0 mg

Microarray protocols

1. RNA extraction from *M. tuberculosis* for microarray analysis

Materials and Methods

[0285]

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- * Trizol (Life Technologies) - formulation of phenol and guanidine thiocyanate.
- * GTC lysis solution containing: 5 M guanidine thiocyanate, 0.5 % N-lauryl sarcosine, 25 mM tri-sodium citrate, 0.1 M 2-mercaptoethanol, and 0.5 % Tween 80.
- * Chloroform
- * Isopropanol
- * 3 M sodium acetate
- * 70 % Ethanol
- * microfuge
- * ribolyser
- * Sterile plasticware-Falcon tubes, screw capped eppendorfs, gilson tips -all RNase free
- * Glassware - baked at 160 °C for at least 16 hours

Method

[0286]

- * Steps performed at Containment level 3; within a Class III microbiological safety cabinet.
- * Remove 10 or 20 ml of culture (10^9 /ml) and immediately add this to 4 volumes of GTC lysis buffer in a plastic specimen pot. Seal the pot tightly.
- * Incubate the cells in GTC lysis buffer for 1 hour at room temperature. Surface decontaminate the plastic pot with 5 % Hycolin for 5 minutes. Transfer the sample to the pass box and place it into a plastic carry tin with a sealable lid. Close the container securely and transport it to a non-toxic cabinet CL3 cabinet.
- * Equally distribute the lysis mixture between Falcon tubes. Place these tubes into centrifuge buckets and seal the buckets tightly. Surface-decontaminate the buckets for 5 minutes with 5 % Hycolin. Then transfer them to the centrifuge (Baird and Tatlock Mark IV refrigerated bench-top centrifuge). Spin the tubes at 3,000 rpm for 30 minutes.
- * Return the unopened buckets to the cabinet. Remove the centrifuge tubes and pour the supernatant into a waste bottle for GTC lysis buffer.
- * Resuspend each pellet in 5 ml of Trizol (formulation of phenol and GTC cat No. 15596-026). The manufacturers guidelines recommend lysing cells by repetitive pipetting. Although this action alone will not lyse *M. tuberculosis*, it is important to completely resuspend the pellet in Trizol.
- * Transfer 1 ml of cells into each FastRNA tube and ribolyse them at power setting 6.5 for 45 seconds.
- * Leave the tubes to incubate at room temperature for 5 minutes.
- * Remove the aqueous layer from each tube and add this to 200 μ l of chloroform in a screw-capped eppendorf tube. Shake each tube vigorously for about 15 seconds. Incubate for 2-3 minutes at room temperature.
- * Spin the tubes at 13,000 rpm for 15 minutes. Following centrifugation, the liquid separates into red phenol/chloroform phase, an interface, and a clear aqueous phase.
- * Carefully remove the aqueous phase and transfer it to fresh eppendorf tubes containing 500 μ l of chloroform/isoamyl alcohol (24:1). Spin the tubes at 13,000 rpm for 15 minutes.
- * Transfer the aqueous phase to eppendorf tubes containing 50 μ l of sodium acetate and 500 μ l of isopropanol.
- * Surface decontaminate the eppendorf tubes with 5% Hycolin for 5 minutes. Remove the tubes from the CL3 laboratory and continue with the procedure in laboratory 157.
- * Steps performed at Containment level 2:
- * Precipitate the RNA at -70 °C for at least 30 minutes (optionally overnight).
- * Spin the precipitated RNA down at 13,000 rpm for 10 minutes. Remove the supernatant and wash the pellet in 70 % ethanol. Repeat centrifugation.
- * Remove the 70 % ethanol and air-dry the pellet. Dissolve the pellet in RNase free water.
- * Freeze the RNA at -70°C to store it.

2. Isolation of genomic DNA from *Mycobacterium tuberculosis* grown in chemostat culture. DNA then used to generate Cy3 or Cy5 labelled DNA for use as a control in microarray experiments

Materials and Methods

[0287]

- Beads 0.5 mm in diameter
- Bead beater
- Bench top centrifuge

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Platform rocker
Heat block
Falcon 50 ml centrifuge tubes
Sorvall RC-5C centrifuge
5 250 ml polypropylene centrifuge pots.
Screw capped eppendorf tubes
Pipettes 1 ml, 200 µl, 10 ml, 5 ml

10 Breaking buffer

[0288]

50 mM Tris HCL pH 8.0
10 mM EDTA
15 100 mM NaCl

Procedure

Mechanical disruption of Mtb cells

[0289]

- 25 * 150 ml of chemostat cells (O. D of 2.5 at 540 nm) are spun down at 15,000 rpm for 15 minutes in 250 ml polypropylene pots using centrifuge Sorvall RC-5C.
* The supernatant is discarded.
* Cells are re-suspended in 5 ml of breaking buffer in a 50 ml Falcon tube and centrifuged at 15,000 rpm for a further 15 minutes.
* The supernatant is removed and additional breaking buffer is added at a volume of 5 ml. Beads are used to disrupt the cells. These are used at a quantity of 1 ml of beads for 1 ml of cells. Place the sample into the appropriate sized
30 chamber. Place in the bead beater and secure the outer unit (containing ice) and process at the desired speed for 30 seconds.
* Allow the beads to settle for 10 minutes and transfer cell lysate to a 50 ml Falcon centrifuge tube
* Wash beads with 2-5 ml of breaking buffer by pipetting washing buffer up and down over the beads.
* Add this washing solution to the lysate in the falcon tube

35 Removal of proteins and cellular components.

[0290]

- 40 * Add 0.1 volumes of 10 % SDS and 0.01 volumes proteinase K.
* Mix by inversion and heat at 55 °C in a heat block for 2-3 hours
* The resulting mix should be homogenous and viscous. If it isn't then add more SDS to bring the concentration up to 0.2 %
* Add an equal volume of phenol/chloroform/Isoamyl alcohol in the ratio: 25/24/1.
45 * Gently mix on a platform rocker until homogenous
* Spin down at 3,000 rpm for 20 minutes
* Remove the aqueous phase and place in a fresh tube
* Extract the aqueous phase with an equal volume of chloroform to remove traces of cell debris and phenol. Chloroform extractions may need to be repeated to remove all the debris.
50 * Precipitate the DNA with 0.3 M sodium acetate and an equal volume of isopropanol.
* Spool as much DNA as you can with a glass rod
* Wash the spooled DNA in 70 % ethanol followed by 100 % ethanol
* Leave to air dry
* Dissolve the DNA in sterile deionised water (500 µl)
55 * Allow DNA to dissolve at 4 °C for approximately 16 hours.
* Add RNase 1 (500U) to the dissolved DNA
* Incubate for 1 hour at 37°C.
* Re-extract with an equal volume of phenol/chloroform followed by a chloroform extraction and precipitate as before

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- * Spin down the DNA at 13,000 rpm
- * Remove the supernatant and wash the pellet in 70 % ethanol
- * Air dry
- * Dissolve in 200-500 µl of sterile water.

3. Preparation of Cy3 or Cy5 labelled DNA from DNA

a) Prepare one Cy3 or one Cy5 labelled DNA sample per microarray slide.

[0291]

Each sample: DNA	2-5 µg
Random primers	(3 µg/µl) 1 µl
H ₂ O	to 41.5 µl

[0292] Heat at 95°C for 5min, snap cool on ice and briefly centrifuge.

Add to each: 10*REact 2 buffer	5 µl
dNTPs (5mM dA/G/TTP, 2mM dCTP)	1 µl
Cy3 OR Cy5 dCTP	1.5 µl
Klenow (5U/µl)	1 µl

Incubate at 37 °C in dark for 90 min.

b) Prehybridise slide

[0293] Mix the prehybridisation solution in a Coplin jar and incubate at 65°C during the labelling reaction to equilibrate.

Prehybridisation: 20*SSC	8.75 ml (3.5*SSC)
20% SDS	250 µl (0.1 % SDS)
BSA (100 mg/ml)	5 ml (10 mg/ml)
H ₂ O	to 50 ml

[0294] Incubate the microarray slide in the pre-heated prehybridisation solution at 65 °C for 20 min. Rinse slide thoroughly in 400 ml H₂O for 1 min followed by rinse in 400 ml propan-2-ol for 1 min and centrifuge slide in 50 ml centrifuge tube at 1,500 rpm for 5 min to dry. Store slide in dark, dust-free box until hybridisation (<1 h).

c) Purify Cy3/Cy5 labelled DNA - Qiagen MinElute Purification

[0295]

- * Combine Cy3 and Cy5 labelled DNA samples in single tube and add 500 µl Buffer PB.
- * Apply to MinElute column in collection tube and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Add 500 µl Buffer PE to MinElute column and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Add 250 µl Buffer PE to MinElute column and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Centrifuge at 13,000 rpm for an additional 1 min to remove residual ethanol.
- * Place the MinElute column into a fresh 1.5 ml tube.
- * Add 10.5 µl H₂O to the centre of the membrane and allow to stand for 1 min.
- * Centrifuge at 13,000 rpm for 1 min.

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4. Preparation of Cy3 or Cy5 label cDNA from RNA

a) Prepare one Cy3 and one Cy5 labelled cDNA sample per microarray slide.

[0296]

Each sample: RNA	2-10 µg
Random primers (3 µg/µl)	1 µl
H ₂ O	to 11 µl

[0297] Heat at 95 °C for 5 min, snap cool on ice and briefly centrifuge.

Add to each: 5*First Strand Buffer	5 µl)
DTT (100 mM)	2.5 µl
dNTPs (5 mM dA/G/TTP, 2 mM dCTP)	2.3 µl
Cy3 OR Cy5 dCTP	1.7 µl
SuperScript II (200 U/µl)	2.5 µl

[0298] Incubate at 25°C in dark for 10 min followed by 42 °C in dark for 90 min.

b) Prehybridise slide

[0299] Mix the prehybridisation solution in a Coplin jar and incubate at 65°C during the labelling reaction to equilibrate.

Prehybridisation:

[0300]

20*SSC	8.75 ml (3.5*SSC)
20 % SDS	250 µl (0.1% SDS)
BSA (100 mg/ml)	5 ml (10 mg/ml)
H ₂ O	to 50 ml

[0301] Incubate the microarray slide in the pre-heated prehybridisation solution at 65 °C for 20 min. Rinse slide thoroughly in 400 ml H₂O for 1 min followed by rinse in 400 ml propan-2-ol for 1 min and centrifuge slide in 50 ml centrifuge tube at 1500 rpm for 5 min to dry. Store slide in dark, dust-free box until hybridisation (<1 h).

c) Purify Cy3/Cy5 labelled cDNA - Qiagen MinElute Purification

[0302]

- * Combine Cy3 and Cy5 labelled DNA samples in single tube and add 250 µl Buffer PB.
- * Apply to MinElute column in collection tube and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Add 500 µl Buffer PE to MinElute column and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Add 250 µl Buffer PE to MinElute column and centrifuge at 13,000 rpm for 1 min.
- * Discard flow-through and place MinElute column back into same collection tube.
- * Centrifuge at 13,000 rpm for an additional 1 min to remove residual ethanol.
- * Place the MinElute column into a fresh 1.5 ml tube.
- * Add 10.5 µl H₂O to the centre of the membrane and allow to stand for 1 min.
- * Centrifuge at 13,000 rpm for 1 min.

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5. Hybridise slide with Cy3/Cy5 labelled cDNA/DNA

[0303] Place the prehybridised microarray slide in the hybridisation cassette and add two 15 µl aliquots of H₂O to the wells in the cassette. Mix resuspended Cy3/Cy5 labelled cDNA sample with hybridisation solution.

Hybridisation:	Cy3/Cy5 labelled cDNA sample	10.5 µl
	20xSSC	3.2 µl (4xSSC)
	2% SDS	2.3 µl (0.3% SDS)

[0304] Heat hybridisation solution at 95 °C for 2 min. Do NOT snap cool on ice but allow to cool slightly and briefly centrifuge. Pipette the hybridisation solution onto the slide at the edge of the arrayed area avoiding bubble formation. Using forceps carefully drag the edge of a cover slip along the surface of the slide towards the arrayed area and into the hybridisation solution at the edge of the array. Carefully lower the cover slip down over the array avoiding any additional movement once in place. Seal the hybridisation cassette and submerge in a water bath at 65°C for 16-20 hours.

Wash slide

[0305] Remove microarray slide from hybridisation cassette and initially wash slide carefully in staining trough of Wash A preheated to 65°C to remove cover slip. Once cover slip is displaced place slide(s) in slide rack and continue agitating in Wash A for a further 2 min.

Wash A:	20xSSC	20 ml (1xSSC)
	20% SDS	1 ml(0.05% SDS)
	H ₂ O	to 400 ml

[0306] Transfer slide(s) to a clean slide rack and agitate in first trough of Wash B for 2 min. Wash in second trough of Wash B with agitation for 2 min.

Wash B (x2): 20xSSC 1.2 ml (0.06xSSC)
H₂O to 400 ml

[0307] Place slide into a 50 ml centrifuge tube and centrifuge at 1500 rpm for 5 mins to dry slide and then scan fluorescence.

Table 2.

Genes induced or up-regulated under nutrient-starving conditions, or under nutrient-starving and oxygen-limiting conditions.		
Gene	Assigned function	SEQ. ID. NO.
Rv0021c	2-nitropropane dioxygenase	1, 2
Rv0029		3, 4
Rv0076c	peptide with a membrane-spanning domain at its C-terminus	5, 6
Rv0111	acetyltransferase	7, 8
Rv0161	oxidoreductase	9, 10
Rv0212c	transcriptional regulator	11, 12
Rv0228	acyl transferase	13, 14
Rv0260c	two-component response regulator	15, 16
Rv0311		17, 18
Rv0322	UDP-glucose dehydrogenase	19, 20
Rv0325		21, 22

(continued)

Genes induced or up-regulated under nutrient-starving conditions, or under nutrient-starving and oxygen-limiting conditions.

Gene	Assigned function	SEQ. ID. NO.
Rv0389	phosphoribosylglycinamide formyltransferase	23, 24
Rv0390		25, 26
Rv0395		27, 28
Rv0480c		29, 30
Rv493c		31, 32
Rv0534c	1,4-dihydroxy-2-naphthoate octaprenyl	33, 34
Rv0557		35, 36
Rv0614		37, 38
Rv0621	peptide containing a membrane-spanning region	39, 40
Rv0622	peptide containing a membrane-spanning region	41, 42
Rv0697	gmc-type oxidoreductase	43, 44
Rv0698		45, 46
Rv0736		47, 48
Rv0751c	3-hydroxyisobutyrate dehydrogenase; methylmalonate semialdehyde dehydrogenase	49, 50
Rv0775		51, 52
Rv0776c		53, 54
Rv0785	dehydrogenase	55, 56
Rv0790c		57, 58
Rv0794c	mercuric reductase; glutathione reductase; dihydrolipoamide dehydrogenase	59, 60
Rv0795	transposase	61, 62
Rv0836c		63, 64
Rv0837c		65, 66
Rv0840c	proline iminopeptidase; prolyl aminopeptidase	67, 68
Rv0849	integral membrane transport protein; quinolone efflux pump	69, 70
Rv0917	glycine betaine transporter	71, 72
Rv978c		73, 74
Rv1051c		75, 76
Rv1056		77, 78
Rv1089		79, 80

(continued)

Genes induced or up-regulated under nutrient-starving conditions, or under nutrient-starving and oxygen-limiting conditions.

Gene	Assigned function	SEQ. ID. NO.
Rv1146	membrane protein	81, 82
Rv1147	phosphatidylethanolamine N-methyltransferase	83, 84
Rv1370c	transposase	85, 86
Rv1371	membrane protein	87, 88
Rv1372	chalcone synthase 2	89, 90
Rv1373	sulfotransferase	91, 92
Rv1429		93, 94
Rv1455		95, 96
Rv1482c		97, 98
Rv1496		99, 100
Rv1526c	glycosyl transferase	101, 102
Rv1528c	PKS-associated protein	103, 104
Rv1552	fumarate reductase flavoprotein	105, 106
Rv1569	8-amino-7-oxononanoate synthase; aminotransferase class-II pyridoxal-phosphate	107, 108
Rv1573	phage phiRv1 protein	109, 110
Rv1577c	bacteriophage HK97 prohead protease; phage phiRv1 protein	111, 112
Rv1670		113, 114
Rv1725c		115, 116
Rv1730	penicillin-binding protein	117, 118
Rv1763	transposase	119, 120
Rv1765c		121, 122
Rv1777	cytochrome p450	123, 124
Rv1806		125, 126
Rv1866	fatty acyl-CoA racemase	127, 128
Rv1917c		129, 130
Rv1939	nitrilotriacetate monooxygenase	131, 132
Rv2013	transposase	133, 134
Rv2027c	histidine kinase response regulator	135, 136
Rv2086	transposase	137, 138
Rv2087	transposase	139, 140
Rv2089c	pepQ; peptidase	141, 142
Rv2091c	peptide containing a transmembrane region	143, 144

(continued)

Genes induced or up-regulated under nutrient-starving conditions, or under nutrient-starving and oxygen-limiting conditions.

Gene	Assigned function	SEQ. ID. NO.
Rv2093c	TatC component of twin-arginine translocation protein export system	145, 146
Rv2105	transposase	147, 148
Rv2168c	transposase	149, 150
Rv2242		151, 152
Rv2282c	LysR transcription regulator	153, 154
Rv2292c		155, 156
Rv2310	excisionase	157, 158
Rv2322c	ornithine aminotransferase	159, 160
Rv2323c		161, 162
Rv2332	malate oxidoreductase	163, 164
Rv2400c	thiosulphate-binding protein	165, 166
Rv2414c		167, 168
Rv2437		169, 170
Rv2478c		171, 172
Rv2486	enoyl-coA hydratase	173, 174
Rv2505c	acyl-CoA synthetase	175, 176
Rv2529	methyltransferase	177, 178
Rv2596		179, 180
Rv2847c	multifunctional enzyme; siroheme synthase	181, 182
Rv3635	transmembrane protein	183, 184
Rv2643	membrane protein	185, 186
Rv2648	transposase	187, 188
Rv2655c		189, 190
Rv2684	transmembrane protein; arsenical pump	191, 192
Rv2687c	regulatory protein	193, 194
Rv2690c	transport protein; permease	195, 196
Rv2800	glutaryl 7-aca acylase	197, 198
Rv2812	transposase	199, 200
Rv2813	secretion pathway protein	201, 202
Rv2835c	sn-glycerol-3-phosphate transport system permease protein	203, 204
Rv2874	integral membrane protein	205, 206
Rv2877c	mercury resistance protein	207, 208
Rv2943	transposase	209, 210

(continued)

Genes induced or up-regulated under nutrient-starving conditions, or under nutrient-starving and oxygen-limiting conditions.

Gene	Assigned function	SEQ. ID. NO.
Rv2998		211, 212
Rv3015c		213, 214
Rv3022c		215, 216
Rv3039c	enoyl-CoA hydratase/isomerase	217, 218
Rv3061c	acyl-CoA dehydrogenase	219, 220
Rv3064c		221, 222
Rv3097c	esterase; lipase	223, 224
Rv3107c	dehydrogenase	225, 226
Rv3162c		227, 228
Rv3178		229, 230
Rv3184	transposase	231, 232
Rv3315c	cytidine deaminase	233, 234
Rv3322c	methyltransferase	235, 236
Rv3351c		237, 238
Rv3352c	oxidoreductase	239, 240
Rv3373	enoyl-CoA hydratase (crotonase)	241, 242
Rv3439c		243, 244
Rv3446c		245, 246
Rv3447c	membrane protein	247, 248
Rv3450c		249, 250
Rv3467		251, 252
Rv3505	acyl-CoA dehydrogenase	253, 254
Rv3540c	lipid-transfer protein	255, 256
Rv3546	acetyl-CoA C-acetyltransferase	257, 258
Rv3550	enoyl-CoA hydratase/isomerase	259, 260
Rv3552		261, 262
Rv3565	aminotransferase	263, 264
Rv3569c	hydrolase	265, 266
Rv3606c	2- amino-4-hydroxy-6-hydroxymethyldihydropterine pyrophosphokinase	267, 268
Rv3637	transposase	269, 270
Rv3660c		271, 272
Rv3745c	Rv3903c 275, 276	273, 274
Rv0039c		277, 278
Rv0903c		279, 280
Rv2745c		281, 282

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 Gln Ala Arg Arg Leu Ser Gln His Phe Ala Arg Val Gly Val Glu Ala
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 Pro Pro Lys Arg Leu Gln Glu Met Leu Leu Gly Ala Pro Ala Ala Asp
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Glu Glu Trp Thr Asp Val Lys Phe Ala Leu Ile Val Thr Gln Leu Asn
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 His Glu Lys Arg Val Ala Lys Phe His Arg Leu Gln Arg Arg Ala Thr
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 Trp Arg Pro Leu Val Trp Leu Gly Thr Ile Ser Tyr Gly Val Tyr Leu
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 Pro Asn Arg Pro Phe Thr Val Ser Val Phe Gly Asp Ser Ile Gly Trp
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 55 Ala Glu Val Leu Arg Val Cys Arg Asp Ala Gly Ala Tyr Val Thr Val
 50 55 60

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 Gly Leu Asp Val Ala Leu Pro Asp Gly Thr Val Leu Arg Arg His Ser
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 Arg Val Arg Arg Asp Asn Thr Gly Tyr Asp Leu Pro Ala Leu Phe Val
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 Glu Pro Ala Leu Tyr Ala Lys Met Met Gly Leu Ile Ala Glu Cys Gly
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 15 Gly Asn Val Ser Ser Glu His Gly Val Gly Ser Arg Lys Arg Ala Tyr
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 25 <213> Mycobacterium tuberculosis

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Arg Gly Phe Leu Pro Ala Val Glu Gly Met Arg Ala Cys Ala Ala Val
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Phe Ala Val Ser Gly Phe Leu Leu Trp Arg Gly His Ala Ala Ala Ala
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Arg Asp Leu Arg Ser His Pro Arg Thr Gly Pro Tyr Leu Arg Ser Arg
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55 Val Ala Arg Ile Met Pro Ala Tyr Val Val Ala Val Val Val Ile Leu

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<400> 19

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55	Ala Phe Met Ala Arg Ala Gly Glu Leu Gly Ala Asp Gln Ala Leu Thr 275 280 285		

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 40 Ala Ala Val Ser Val Asp Ala Leu Asp Ala Val Glu Gln Ala Asp Gly
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<213> Mycobacterium tuberculosis

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Ala Glu Val Ala Thr Glu Ala Gly Ile Thr Pro Ala Tyr Asn Val Leu
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<210> 26

30 <211> 420

<212> DNA

<213> Mycobacterium tuberculosis

35 <400> 26

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40 ttgtcgagcc ttggtcgtga agtgggtctat gtcgaatggg cgacgtccga cgggacgcac 180

aacgacaact tcctcgccga gttgcgggac cgcaccccg cggacgctga tcagcacgag 240

cggcccgtta ttttcttgtg tcgctccggt aaccgctcca tcggcgcggc cgaggtcgcg 300

45 accgaggcgg gcatcacgcc ggcctataac gtgctggacg gcttcgaagg gcatctcgac 360

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50 <211> 134

<212> PRT

<213> Mycobacterium tuberculosis

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<400> 27

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Gly Lys Pro Arg Ala Trp Gly Pro Gln Glu Ser Gly Trp Arg Ala Trp
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Phe Gly Gly Lys Ile Val Asp Gly Leu Cys Glu Val Leu Asp Glu His
35 40 45

Leu Ala Val Arg Arg Arg Gly Val Pro Ala Ala Ile Gly Cys Val Pro
50 55 60

Trp Leu Ser Ser Glu Ala Val Ala Glu Thr Leu Leu Ala Leu Ser Val
65 70 75 80

Phe Cys Val Val Ile Asp Lys Gly Thr Ser Phe Pro Ser Arg Leu Arg
85 90 95

Asn Pro Asp Lys Gly Phe Pro Asn Val Ala Leu Leu Arg Leu Arg Asp
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Met Ala Pro Ser Glu His Gly Ser Arg Cys Ser Ser Ala Arg Gly Arg
115 120 125

Leu Cys Leu Ser Met Ser
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<210> 28

<211> 402

<212> DNA

<213> Mycobacterium tuberculosis

<400> 28

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ctctgcgagg tactcgacga gcacctcgcg gtgcggcgtc gtggtgttcc agccgcgatac 180

ggetgcgtgc cctggctgag tagcgaggcg gtcgccgaga cgctgctcgc attgagcgtc 240

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<210> 29

<211> 340

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<212> PRT

<213> Mycobacterium tuberculosis

<400> 29

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Pro Gly Ala Ser Trp Ile Ala Arg Leu Leu Arg Ala Pro Val Arg Arg
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Ala Arg Arg Arg Ala Gln Ala Gly Leu Pro Gly Ser Cys Ala Arg Arg
35 40 45

20

Cys Gly Ala Leu Val Ala Gly Pro Arg Leu Ala Arg Met Arg Ile Ala
50 55 60

Leu Ala Gln Ile Arg Ser Gly Thr Asp Pro Ala Ala Asn Leu Gln Leu
65 70 75 80

25

Val Gly Lys Tyr Ala Gly Glu Ala Ala Thr Ala Gly Ala Gln Leu Val
85 90 95

30

Val Phe Pro Glu Ala Thr Met Cys Arg Leu Gly Val Pro Leu Arg Gln
100 105 110

Val Ala Glu Pro Val Asp Gly Pro Trp Ala Asn Gly Val Arg Arg Ile
115 120 125

35

Ala Thr Glu Ala Gly Ile Thr Val Ile Ala Gly Met Phe Thr Pro Thr
130 135 140

40

Gly Asp Gly Arg Val Thr Asn Thr Leu Ile Ala Ala Gly Pro Gly Thr
145 150 155 160

Pro Asn Gln Pro Asp Ala His Tyr His Lys Ile His Leu Tyr Asp Ala
165 170 175

45

Phe Gly Phe Thr Glu Ser Arg Thr Val Ala Pro Gly Arg Glu Pro Val
180 185 190

50

Val Val Val Val Asp Gly Val Arg Val Gly Leu Thr Val Cys Tyr Asp
195 200 205

Ile Arg Phe Pro Ala Leu Tyr Thr Glu Leu Ala Arg Arg Gly Ala Gln
210 215 220

55

Leu Ile Ala Val Cys Ala Ser Trp Gly Ser Gly Pro Gly Lys Leu Glu

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15	Ser Pro Leu Gly Glu Val Val Val Ser Ala Gly Thr Gln Pro Gln Leu 290 295 300				
	Leu Val Ala Asp Ile Asp Val Asp Asn Val Ala Ala Ala Arg Asp Arg 305 310 315 320				
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 15 <213> Mycobacterium tuberculosis
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 35 40 45
 30 Leu Trp Val His Cys Glu Thr Val Ala Pro Thr Thr Gly Gly Pro Tyr
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 Ala His Gly Trp Val Thr Trp Phe Pro Pro Asp Ala Pro Pro Gly Thr
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 85 90 95
 40 Phe Asp Ile Ala Gly Val Arg Met Ala Pro Ala Glu Leu Thr Gly Arg
 100 105 110
 Thr Arg Ser Leu Ala Trp Glu Leu Ser Trp Lys Asp Thr Ala Ala Pro
 115 120 125
 45 Leu Trp Thr Phe Pro Arg Val Ala Trp Glu Arg Glu Leu Leu Pro Gly
 130 135 140
 50 Ala Gln Val Val Ile Ala Pro Thr Ala Val Phe Ala Gly Ser Leu Ala
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 Val Gly Glu Thr Thr His Arg Val Asp Ser Trp Arg Gly Ser Val Ala
 165 170 175
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His Ile Tyr Gly His Gly Asn Ala Lys Arg Trp Gly Trp Ile His Ala
180 185 190

5 Asp Leu Gly Asp Gly Asp Val Leu Glu Val Val Thr Ala Val Ser His
195 200 205

Lys Pro Gly Leu Arg Arg Leu Ala Pro Leu Ala Phe Val Arg Phe Arg
210 215 220

10 Ile Asp Gly Lys Asp Trp Pro Ala Ser Pro Leu Pro Ser Leu Arg Met
225 230 235 240

15 Arg Thr Thr Leu Gly Val Arg His Trp Gln Leu Glu Gly Arg Ile Gly
245 250 255

Gly Arg Glu Ala Leu Ile Arg Val Asp Gln Pro Pro Glu Arg Cys Val
260 265 270

20 Ser Leu Gly Tyr Thr Asp Pro Asp Gly Ala Lys Ala Val Cys Thr Asn
275 280 285

25 Thr Glu Gln Ala Asp Ile His Ile Glu Leu Gly Gly Arg His Trp Ser
290 295 300

Val Leu Gly Thr Gly His Ala Glu Val Gly Leu Arg Gly Thr Ala Ala
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30 Pro Ala Ile Lys Glu Gly Thr Pro Ala
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<210> 32

35 <211> 987

<212> DNA

40 <213> Mycobacterium tuberculosis

<400> 32

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tcggatccac tgacgcgtac cggcctatgg gtgcactgtg agacggtggc cccgacgacc 180

ggcggggcct acgcgcacgg ctgggtgacc tggtttccac cggatgcccc gccgggcacc 240

50 gacgccttcg gccccagcc cgcccaaccc gcggccggcc ccgcctggtt cgacatcgcc 300

ggtgtacgaa tggcgccagc ggagctgacc ggacgtaccc gatcactcgc atgggagctg 360

tcttggaagg acaccgcggc gccactgtgg acgtttccto gcgtggcctg ggagcgcgag 420

55 ttgctgcccg gcgcccaggt ggtgatcgca cccaccgcg tcttcgctgg ctcttgggcc 480

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<211> 292

<212> PRT

<213> Mycobacterium tuberculosis

<400> 33

Val Ala Ser Phe Ala Gln Trp Val Ser Gly Ala Arg Pro Arg Thr Leu
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Pro Asn Ala Ile Ala Pro Val Val Ala Gly Thr Gly Ala Ala Ala Trp
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Leu His Ala Ala Val Trp Trp Lys Ala Leu Leu Ala Leu Ala Val Ala
35 40 45

Val Ala Leu Val Ile Gly Val Asn Tyr Ala Asn Asp Tyr Ser Asp Gly
50 55 60

Ile Arg Gly Thr Asp Asp Asp Arg Val Gly Pro Val Arg Leu Val Gly
65 70 75 80

Ser Arg Leu Ala Thr Pro Arg Ser Val Leu Thr Ala Ala Met Thr Ser
85 90 95

Leu Ala Leu Gly Ala Leu Ala Gly Leu Val Leu Ala Leu Leu Ser Ala
100 105 110

Pro Trp Leu Ile Ala Val Gly Ala Ile Cys Ile Ala Gly Ala Trp Leu
115 120 125

Tyr Thr Gly Gly Ser Lys Pro Tyr Gly Tyr Ala Gly Phe Gly Glu Leu
130 135 140

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	Ala	Val	Phe	Val	Phe	Phe	Gly	Pro	Val	Ala	Val	Leu	Gly	Thr	Gln	Tyr	
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<210> 35

<211> 378

<212> PRT

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<213> Mycobacterium tuberculosis

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<400> 35

Val Cys Gly Val Arg Val Ala Ile Val Ala Glu Ser Phe Leu Pro Gln
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Val Asn Gly Val Ser Asn Ser Val Val Lys Val Leu Glu His Leu Arg
 20 25 30

Arg Thr Gly His Glu Ala Leu Val Ile Ala Pro Asp Thr Pro Pro Gly
 35 40 45

35

Glu Asp Arg Ala Glu Arg Leu His Asp Gly Val Arg Val His Arg Val
 50 55 60

40

Pro Ser Arg Met Phe Pro Lys Val Thr Thr Leu Pro Leu Gly Val Pro
 65 70 75 80

Thr Phe Arg Met Leu Arg Ala Leu Arg Gly Phe Asp Pro Asp Val Val
 85 90 95

45

His Leu Ala Ser Pro Ala Leu Leu Gly Tyr Gly Gly Leu His Ala Ala
 100 105 110

Arg Arg Leu Gly Val Pro Thr Val Ala Val Tyr Gln Thr Asp Val Pro
 115 120 125

50

Gly Phe Ala Ser Ser Tyr Gly Ile Pro Met Thr Ala Arg Ala Ala Trp
 130 135 140

55

Ala Trp Phe Arg His Leu His Arg Leu Ala Asp Arg Thr Leu Ala Pro
 145 150 155 160

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5 Ser Thr Ala Thr Met Glu Ser Leu Ile Ala Gln Gly Ile Pro Arg Val
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 His Arg Trp Ala Arg Gly Val Asp Val Gln Arg Phe Ala Pro Ser Ala
 180 185 190
 10 Arg Asn Glu Val Leu Arg Arg Arg Trp Ser Pro Asp Gly Lys Pro Ile
 195 200 205
 Val Gly Phe Val Gly Arg Leu Ala Pro Glu Lys His Val Asp Arg Leu
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 15 Thr Gly Leu Ala Ala Ser Gly Ala Val Arg Leu Val Ile Val Gly Asp
 225 230 235 240
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 245 250 255
 Thr Gly Ala Arg Tyr Gly Lys Glu Leu Ala Glu Ala Tyr Ala Ser Met
 260 265 270
 25 Asp Val Phe Val His Ser Gly Glu His Glu Thr Phe Cys Gln Val Val
 275 280 285
 30 Gln Glu Ala Leu Ala Ser Gly Leu Pro Val Ile Ala Pro Asp Ala Gly
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 Gly Pro Arg Asp Leu Ile Thr Pro His Arg Thr Gly Leu Leu Leu Pro
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 35 Val Gly Glu Phe Glu His Arg Leu Pro Asp Ala Val Ala His Leu Val
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 40 His Glu Arg Gln Arg Tyr Ala Leu Ala Ala Arg Arg Ser Val Leu Gly
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 50 <210> 36
 <211> 1134
 <212> DNA
 55 <213> Mycobacterium tuberculosis

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 Ser Met Gly His Val Arg Pro Gly Phe Ser Pro Arg Leu Gly Ser His
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 55 Arg Thr Leu Arg Pro Arg Trp Pro Pro Tyr Ala Ala Ala Ser Arg Gly

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	Gln Arg Ala Ile Gln Leu Val Val Ser Glu Gln Glu Val Ala Ser Lys 130					
20	Ile Thr Ala Ala Thr Ala Gly Ile Ala Thr Thr Thr Phe Pro Glu Thr 145					
25	Pro Ser Ile Asp Asp Thr Ile Ile Gly Asn Asp Asn Arg Asp Thr Gly 165					
	Val Arg Leu Val Asp Val Lys Gln Asp Gly Gly Thr Ser Pro Pro Pro 180					
30	Pro Phe Ala Pro Trp Asp Thr Pro Asp Gly Thr Pro Pro Pro Gly Thr 195					
35	Gly Leu Ser Pro Thr Leu Gln Gln Met Ile Leu Gly Gly Asp Pro Ala 210					
	Asn Leu Thr Gly Gln Gly Leu Ala Asp Asn Val Gln Arg Phe Val Gln 225					
40	Ser Leu Pro Ala Asn Asp Pro Asn Thr Ala Trp Leu Arg Gly Gln Val 245					
45	Ala Asp Leu Gln Ala His Val Ala Asp Ile Glu Tyr Ala Arg Thr His 260					
	Cys Ser Thr Asn Asp Trp Ile Asp Arg Thr Ala Gln Phe Ala Ser Gly 275					
50	Ala Ile Val Phe Ser Ile Gly Val Leu Thr Ala Glu Thr Gly Ala Gly 290					
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Ser Leu Leu Gln Cys Leu Val Gly Ser Lys
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<211> 990
<212> DNA
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<213> Mycobacterium tuberculosis

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<213> Mycobacterium tuberculosis

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 Gln Gly His Val Asn Ala Trp Asp Tyr Arg Phe Cys Glu Arg Cys Gly
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 Ser Pro Ile Gly Val Val Pro Trp Pro Ser Glu Glu Ser Gly Thr Arg
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 Gln Thr Ala Pro Ala Arg Ser Phe Val Pro Leu Val Val Leu Ala Ala
 65 70 75 80
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 Thr Leu Leu Val Val Ala Val Val Val Thr Ala Val Gly Tyr Ala Val
 85 90 95
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 Thr Arg Pro Ala Arg Asn Asp Arg Glu Glu Pro Ser Ser Ala Arg Gly
 100 105 110
 Ala Ala Thr Thr Gly Val Pro Phe Ala Gln Ala Glu Ala Ala Ser Cys
 115 120 125
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 Pro Asp Asp Pro Val Leu Glu Ala Glu Ser Ile Asp Leu Thr Ser Asp
 130 135 140
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 Gly Leu Ala Val Ser Ala Ala Phe Met Ser Ala Cys Ala Gly Gly Asp
 145 150 155 160
 Val Glu Ser Asn Ser Ala Leu Glu Val Thr Val Ala Asp Gly Arg Arg
 165 170 175
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 Asp Val Ala Ala Gly Ser Phe Asp Phe Ser Ala Asp Pro Leu Arg Ile
 180 185 190
 40
 Glu Pro Gly Val Pro Ala Arg Arg Thr Leu Val Phe Pro Pro Gly Met
 195 200 205
 Tyr Trp Arg Thr Pro Asp Met Leu Ser Gly Ala Pro Ala Leu Ala Ala
 210 215 220
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 Thr Arg Lys Gly Arg Ser Asp Arg Ser Ala Ala Arg Gly Gly Ser Ala
 225 230 235 240
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 Arg Thr Thr Met Val Ala Ala Ala Ser Ala Ala Pro Ala Tyr Gly Ser
 245 250 255
 Ile Asn Ala Val Ala Gly Ala Val Leu Val Glu Leu Arg Asp Ser Asp
 260 265 270
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 Phe Pro Tyr Val Arg Val Gly Ile Ala Asn Arg Trp Val Pro Gln Val
 275 280 285

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5 Ser Ser Lys Arg Val Gly Leu Val Ala Ala Gly Lys Thr Trp Thr Ser
 290 295 300
 Ala Asp Ile Leu Arg Asp His Leu Ala Leu Arg Gln Arg Phe Gly Gly
 305 310 315 320
 10 Ala Arg Leu Val Trp Ser Gly His Trp Thr Thr Phe Ser Gly Pro Asp
 325 330 335
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 15 Asn Arg
 20 <210> 40
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 <212> DNA
 25 <213> Mycobacterium tuberculosis
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 tacaggttct gtgagcgggtg cggctcgccg atcggcggtg tgccctggcc gtcggaggaa 180
 tcaggcacac gccagacggc gccgcgcga tccttcgtcc ccctcgctgt cctcgcggcg 240
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 55 gcccgctgg tgtggtcggg gcaactggacc accttcagcg gacccgattt ctgggtgacg 1020

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1062

<210> 41

<211> 341

<212> PRT

<213> Mycobacterium tuberculosis

<400> 41

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Cys Gly Ala Glu Leu Ala Asp Pro Thr Arg Cys Gly Ala Cys Gly Ala
35 40 45

Tyr Lys Ile Gly Ser Thr Trp His Arg Thr Thr Thr Pro Thr Val Gly
50 55 60

Ala Ala Thr Thr Ala Thr Gly Trp Arg Pro Asp Pro Thr Gly Arg His
65 70 75 80

Glu Gly Arg Tyr Phe Val Ala Gly Gln Pro Thr Asp Leu Val Arg Glu
85 90 95

Gly Asp Ala Glu Ala Val Asp Pro Leu Gly Gln Gln Gln Leu Asp Gln
100 105 110

Ser Gly Ala Val Gly Val Ser Pro Ser Ala Val Ser Gly Trp Val Arg
115 120 125

Ser Gly His Arg Arg Leu Trp Trp Ala Leu Ala Gly Val Val Ala Phe
130 135 140

Leu Gly Leu Val Gly Ala Gly Val Val Gly Thr Leu Phe Leu Asn Arg
145 150 155 160

Asp Arg Glu Ser Ile Asp Asp Lys Tyr Leu Ala Ala Leu Arg Arg Ser
165 170 175

Gly Leu Thr Gly Glu Phe Asn Ser Asp Ala Asn Ala Ile Ala Arg Gly
180 185 190

Lys Gln Val Cys Arg Gln Leu Gln Asp Gly Gly Glu Gln Gln Gly Met
195 200 205

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	Pro Val Asp Gln Val Ala Val Gln Tyr Tyr Cys Pro Gln Phe Ser Asp	
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	225 230 235 240	
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	Ser Gly Cys Ser Gly Ser Ala Gly Tyr Ala Asp Ile Asp Arg Gly Thr	
	260 265 270	
15	Gln Val Thr Val Lys Asn Gly Gln Gly Asp Ile Leu Ala Thr Ala Phe	
	275 280 285	
	Leu Gln Ala Gly Gln Gly Gly Arg Phe Leu Cys Thr Phe Pro Phe Ser	
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	Phe Glu Ile Thr Glu Gly Glu Asp Arg Tyr Val Val Ser Val Ser Arg	
	305 310 315 320	
25	Arg Gly Glu Met Ser Tyr Ser Phe Ala Asp Leu Lys Ala Asn Gly Leu	
	325 330 335	
	Ser Leu Val Leu Gly	
30	340	
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	<211> 1023	
35	<212> DNA	
	<213> Mycobacterium tuberculosis	
40	<400> 42	
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	ccgacggtcg gcgcgcgcgac gacggcaacg ggatggcgac ccgatcccac cggtcgccac	240
	gagggacgct acttcgtcgc cgggcagccg accgacctcg ttcgcgaggc cgacgccgaa	300
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	tcagcgggtg cgggggtggg gcgttctggg caccgtcgac tgtggtgggc gcttgccggc	420
	gtggtggcgt ttctcgggct ggtgggagcc ggtgtcgctg ggacgctgtt cctgaatcga	480
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	gagttcaact ccgacgcgaa cgccatcgcc cgcggcaagc aggtgtgccg ccagttgcaa	600

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<210> 43

<211> 479

20 <212> PRT

<213> Mycobacterium tuberculosis

25 <400> 43

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30 Ser Ala Gly Ser Val Val Ala Glu Arg Leu Ser Met Asp Ser Ser Cys
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Val Val Thr Val Leu Glu Ala Gly Pro Gly Leu Ala Asp Pro Gly Leu
 35 40 45

Leu Ala Gln Thr Ala Asn Gly Leu Gln Leu Pro Ile Gly Ala Gly Ser
 50 55 60

40 Pro Leu Val Glu Arg Tyr Arg Thr Arg Leu Thr Asp Arg Pro Val Arg
 65 70 75 80

His Leu Pro Ile Val Arg Gly Ala Thr Val Gly Gly Ser Gly Ala Ile
 45 85 90 95

Asn Gly Gly Tyr Phe Cys Arg Gly Leu Pro Ser Asp Phe Asp Arg Ala
 100 105 110

50 Ser Ile Pro Gly Trp Ala Trp Ser Asp Val Leu Glu His Phe Arg Ala
 115 120 125

Ile Glu Thr Asp Leu Asp Phe Glu Thr Pro Val His Gly Arg Ser Gly
 55 130 135 140

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	Pro	Ile	Pro	Val	Arg	Arg	Thr	His	Glu	Met	Thr	Gly	Ile	Thr	Glu	Ser	145			150		155					160
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15	Pro	Ala	Leu	Gly	Arg	Pro	Asn	Leu	Thr	Leu	Leu	Ala	Arg	Thr	Arg	Ala			210			215			220		
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25	Ala	Gly	Ala	Ile	Gln	Ser	Ala	His	Leu	Leu	Met	Leu	Ser	Gly	Val	Gly				260			265			270	
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30	Pro	Val	Gly	Met	Gly	Cys	Ser	Asp	His	Pro	Glu	Trp	Val	Met	Pro	Thr			290			295			300		
35	Asn	Trp	Ala	Val	Ala	Val	Asp	Arg	Pro	Val	Leu	Glu	Val	Leu	Leu	Ser			305			310			315		320
	Thr	His	Asp	Gly	Ile	Glu	Ile	Arg	Pro	Tyr	Thr	Gly	Gly	Phe	Val	Ala				325			330			335	
40	Met	Thr	Gly	Asp	Gly	Thr	Ala	Gly	His	Arg	Asp	Trp	Pro	His	Ile	Gly				340			345			350	
45	Val	Ala	Leu	Met	Gln	Pro	Arg	Ala	Arg	Gly	Arg	Ile	Thr	Leu	Val	Ser				355			360			365	
	Ser	Asp	Pro	Gln	Ile	Pro	Val	Arg	Ile	Glu	His	Arg	Tyr	Asp	Ser	Glu			370			375			380		
50	Pro	Ala	Asp	Val	Ala	Ala	Leu	Arg	Gln	Gly	Ser	Ala	Leu	Ala	His	Glu			385			390			395		400
55	Leu	Cys	Gly	Ala	Ala	Thr	Arg	Ile	Gly	Pro	Ala	Val	Trp	Ala	Thr	Ser				405			410			415	

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Gln His Leu Cys Gly Ser Ala Pro Met Gly Thr Asp Asp Asp Pro Arg
420 425 430

5
Ala Val Val Asp Pro Arg Cys Arg Val Arg Gly Ile Glu Asn Leu Trp
435 440 445

10
Val Ile Asp Gly Ser Val Leu Pro Ser Ile Thr Ser Arg Gly Pro His
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Ala Thr Ile Val Met Leu Gly His Arg Ala Ala Glu Phe Val Gln
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<211> 1437
<212> DNA
20 <213> Mycobacterium tuberculosis

<400> 44
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 10 <210> 45
 <211> 203
 <212> PRT
 15 <213> Mycobacterium tuberculosis
 <400> 45
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 20 25 30
 25 Phe Arg Pro Gln Asn Thr Leu Gly Asp Gly Ala Asn Gly Leu Pro Leu
 35 40 45
 30 Ala Val Cys Thr Thr Thr Ala His Thr Cys His Thr Ser His Thr His
 50 55 60
 Pro Ser Arg Trp Thr Pro Asn Pro Val Pro Ala Thr Lys Gly Val Pro
 65 70 75 80
 35 Ala Gly Leu Val Gln Ala Thr Phe Ile Ile Glu Asn Leu Asp Pro Gly
 85 90 95
 40 Asn Asn Asp Thr Pro Thr Pro Pro Thr Pro Lys Leu Arg Leu Ala Arg
 100 105 110
 Lys Pro Gly His His Arg Arg Ser Glu Tyr Asp Ala Asp Ser Val Leu
 115 120 125
 45 Arg Arg Lys Asp Thr Ser Arg Arg Cys Val Gln Ala Asp Asp Val Arg
 130 135 140
 50 Cys Val Gln Leu Val Gln Asp Pro Arg Arg Gly Arg Val Glu Leu Gly
 145 150 155 160
 Gly Tyr Arg Ala Glu Leu Thr Val Gly Arg Arg Ala Ala Val Asn Cys
 165 170 175
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Gln Arg Pro Gln Tyr Gly Ala Asp Gly Trp Pro Val Arg Leu Gly Cys
180 185 190

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<210> 46

<211> 609

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<212> DNA

<213> Mycobacterium tuberculosis

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<400> 46

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tcccatactc acccatcgcg gtggacccca aaccagtgcc cggccaccaa gggcgcccc 240

gctggattgg tgcaagcaac cttcatcatc gaaaaccttg accccggcaa caacgacacg 300

25

ccgaccccc ctacaccaa actgcgatta gcccgaaaac ctgggcacca taggcgatct 360

gaatacgatg cggattcggt gctgcgagaa aaggatacat cgcgccgatg cgtccaggcg 420

gatgacgtcc gatgcgtgca gctgggtccag gatcccgagg cgggacgtgt cgaactcggc 480

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ggttaccgag ccgagcttac tgttgccga cgggcggcgg tgaattgcca acgcccga 540

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40

<213> Mycobacterium tuberculosis

<400> 47

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Arg Glu Val Ser Thr Gly Asp Asp His His Tyr Ala Met Trp Asp Ala
20 25 30

50

Ala Tyr Val Leu Gly Ala Leu Ser Ala Ala Asp Arg Arg Glu Phe Glu
35 40 45

55

Ala His Leu Ala Gly Cys Pro Glu Cys Arg Gly Ala Val Thr Glu Leu
50 55 60

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5 Cys Gly Val Pro Ala Leu Leu Ser Gln Leu Asp Arg Asp Glu Val Ala
65 70 75 80

Ala Ile Ser Glu Ser Ala Pro Thr Val Val Ala Ser Gly Leu Ser Pro
85 90 95

10 Glu Leu Leu Pro Ser Leu Leu Ala Ala Val His Arg Arg Arg Arg Arg
100 105 110

Thr Arg Leu Ile Thr Trp Val Ala Ser Ser Ala Ala Ala Val Leu
115 120 125

15 Ala Ile Gly Val Leu Val Gly Val Gln Gly His Ser Ala Ala Pro Gln
130 135 140

20 Arg Ala Ala Val Ser Ala Leu Pro Met Ala Gln Val Gly Thr Gln Leu
145 150 155 160

Leu Ala Ser Thr Val Ser Ile Ser Gly Glu Pro Trp Gly Thr Phe Ile
165 170 175

25 Asn Leu Arg Cys Val Cys Leu Ala Pro Pro Tyr Ala Ser His Asp Thr
180 185 190

30 Leu Ala Met Val Val Val Gly Arg Asp Gly Ser Gln Thr Arg Leu Ala
195 200 205

Thr Trp Leu Ala Glu Pro Gly His Thr Ala Thr Pro Ala Gly Ser Ile
210 215 220

35 Ser Thr Pro Val Asp Gln Ile Ala Ala Val Gln Val Val Ala Ala Asp
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245 250

<210> 48

45 <211> 750

<212> DNA

<213> Mycobacterium tuberculosis

50 <400> 48

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55 gcggccgacc gccgcgaatt cgaagcgcac ctggccgggt gccccgaatg ccggggggcc 180

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 25 <213> Mycobacterium tuberculosis
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 35 40 45
 40 Arg Ser Ala Pro Glu Ala Val Ala Glu Ala Asp Val Val Ile Thr Met
 50 55 60
 Leu Pro Thr Gly Glu Val Val Arg Arg Cys Tyr Thr Asp Val Leu Ala
 65 70 75 80
 45 Ala Ala Arg Pro Ala Thr Leu Phe Ile Asp Ser Ser Thr Ile Ser Val
 85 90 95
 50 Thr Asp Ala Arg Glu Val His Ala Leu Ala Glu Ser His Gly Met Leu
 100 105 110
 Gln Leu Asp Ala Pro Val Ser Gly Gly Val Lys Gly Ala Ala Ala Ala
 115 120 125
 55 Thr Leu Ala Phe Met Val Gly Gly Asp Glu Ser Thr Leu Arg Arg Ala

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	130		135		140	
5	Arg 145	Pro Val	Leu Glu	Pro 150	Met Ala	Gly Lys Ile 155
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	Ala Gly	Ala Gly	Gln 165	Ala Ala	Lys Val	Cys Asn Asn Met Val Leu Ala 175
10	Val Gln	Gln Ile	Ala Ile	Ala Glu	Ala 185	Phe Val Leu Ala Glu Lys Leu 190
15	Gly Leu	Ser 195	Ala Gln	Ser Leu	Phe 200	Asp Val Ile Thr Gly Ala Thr Gly 205
	Asn 210	Cys Trp	Ala Val	His Thr	Asn 215	Cys Pro Val Pro 220
20						Gly Phe Ser Thr Ala Leu 240
	Thr 225	Ser Pro	Ala Asn	Asn Asp	Phe Lys	Pro Gly Phe Ser Thr Ala Leu 240
25	Met Asn	Lys Asp	Leu 245	Gly Leu	Ala Met	Asp 250
						Ala Val Ala Ala Thr Gly 255
	Ala Thr	Ala Pro	Leu Gly	Ser His	Ala 265	Ala Asp Ile Tyr Ala Lys Phe 270
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<211> 207

<212> PRT

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<400> 51

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Ala Val Arg His Arg Ala Val Ala Arg Arg Ala Gly Leu Pro Leu Ala
35 40 45

Ser Thr Thr Tyr Tyr Phe Ser Ser Leu Asp Asp Leu Ile Ala Arg Ala
50 55 60

Val Glu His Ile Gly Met Ile Glu Val Ala Gln Leu Arg Ala Arg Val
65 70 75 80

Ser Ala Leu Ser Arg Arg Arg Arg Gly Pro Glu Thr Thr Ala Val Val
85 90 95

Leu Val Asp Leu Leu Val Gly Glu Met Ser Ser Pro Gly Leu Ala Glu
100 105 110

Gln Leu Ile Ser Arg Tyr Glu Arg His Ile Ala Cys Thr Arg Leu Pro
115 120 125

Asp Leu Arg Glu Ser Met Arg Arg Ser Leu Arg Gln Arg Ala Glu Ala
130 135 140

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Val Ala Glu Ala Ile Glu Arg Ser Gly Arg Ser Ala Gln Ile Glu Leu
145 150 155 160

5 Val Cys Thr Leu Ile Cys Ala Val Asp Gly Ser Val Val Ser Ala Leu
165 170 175

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180 185 190

Leu Ile Asp Val Leu Ala Pro Val Asp Gln Arg Pro Val Pro Phe
195 200 205

15 <210> 52
<211> 621
<212> DNA
20 <213> Mycobacterium tuberculosis

<400> 52
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45 <211> 259
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 5 Ala Arg Asp Asp Ala Ser Val Leu Ala Ala Leu Arg Pro Tyr Val Val
 35 40 45
 Gly Asp Cys Leu Val Ala Phe Asp Ala Pro Leu Val Val Ala Asn Arg
 50 55 60
 10 Thr Gly Gln Arg Pro Ala Glu Ala Ala Leu Asn Arg Asp Phe Arg Gln
 65 70 75 80
 15 Phe Glu Ala Gly Ala Tyr Pro Ala Asn Thr Glu Lys Pro Glu Phe Ala
 85 90 95
 Asp Val Pro Arg Ala Ala Arg Leu Ala Arg Gln Leu Ala Leu Asp Met
 100 105 110
 20 Asp Pro Leu Ser Ser Ala Thr Arg Arg Ala Ile Glu Val Tyr Pro His
 115 120 125
 25 Pro Ala Thr Val Ala Leu Phe Arg Leu Pro Arg Ala Leu Lys Tyr Lys
 130 135 140
 Ala Lys Pro Gly Arg Ser Val Asp Leu Leu Lys Ser Glu Leu Leu Arg
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 30 Leu Met Asp Gly Val Glu Gly Leu Ala Gln Ala Gly Val Arg Met Gln
 165 170 175
 35 Val Ala Gly Gln Pro Asp Trp Val Ser Leu Arg Arg Gln Val Thr Val
 180 185 190
 Ala Gln Arg Lys Ser Asp Leu Arg Ala Ala Glu Asp Pro Ile Asp Ala
 195 200 205
 40 Val Val Cys Ala Tyr Val Ala Leu Tyr Ala Gln Arg Arg Pro Ala Asp
 210 215 220
 45 Val Thr Ile Tyr Gly Asp Phe Thr Thr Gly Tyr Ile Val Thr Pro Ser
 225 230 235 240
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<211> 777

<212> DNA

5 <213> Mycobacterium tuberculosis

<400> 54

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cggcccgcgg atgtcacgat ctatggggac ttcaccaccg ggtacattgt cagcccgctc 720
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<211> 566

35 <212> PRT

<213> Mycobacterium tuberculosis

40 <400> 55

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Gly Leu Val Ala Ala Cys Glu Leu Ala Asp Arg Gly Leu Arg Val Leu
35 40 45
50 Ile Leu Asp Gln Glu Asn Arg Ala Asn Val Gly Gly Gln Ala Phe Trp
50 55 60
55 Ser Phe Gly Gly Leu Phe Leu Val Asn Ser Pro Glu Gln Arg Arg Leu
65 70 75 80

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Gly Ile Arg Asp Ser His Glu Leu Ala Leu Gln Asp Trp Leu Gly Thr
 85 90 95
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 Ala Ala Phe Asp Arg Pro Glu Asp Tyr Trp Pro Glu Gln Trp Ala His
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 Ala Tyr Val Asp Phe Ala Ala Gly Glu Lys Arg Ser Trp Leu Arg Ala
 115 120 125
 Arg Gly Leu Lys Ile Phe Pro Leu Val Gly Trp Ala Glu Arg Gly Gly
 130 135 140
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 Tyr Asp Ala Gln Gly His Gly Asn Ser Val Pro Arg Phe His Ile Thr
 145 150 155 160
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 Trp Gly Thr Gly Pro Ala Leu Val Asp Ile Phe Val Arg Gln Leu Arg
 165 170 175
 Asp Arg Pro Thr Val Arg Phe Ala His Arg His Gln Val Asp Lys Leu
 180 185 190
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 Ile Val Glu Gly Asn Ala Val Thr Gly Val Arg Gly Thr Val Leu Glu
 195 200 205
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 Pro Ser Asp Glu Pro Arg Gly Ala Pro Ser Ser Arg Lys Ser Val Gly
 210 215 220
 Lys Phe Glu Phe Arg Ala Ser Ala Val Ile Val Ala Ser Gly Gly Ile
 225 230 235 240
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 Gly Gly Asn His Glu Leu Val Arg Lys Asn Trp Pro Arg Arg Met Gly
 245 250 255
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 Arg Ile Pro Lys Gln Leu Leu Ser Gly Val Pro Ala His Val Asp Gly
 260 265 270
 Arg Met Ile Gly Ile Ala Gln Lys Ala Gly Ala Ala Val Ile Asn Pro
 275 280 285
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 Asp Arg Met Trp His Tyr Thr Glu Gly Ile Thr Asn Tyr Asp Pro Ile
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 Trp Pro Arg His Gly Ile Arg Ile Ile Pro Gly Pro Ser Ser Leu Trp
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 325 330 335
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 Asp Thr Leu Gly Thr Leu Glu Tyr Ile Thr Lys Ser Gly His Asp Tyr

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	<211>	1698															
	<212>	DNA															
55	<213>	Mycobacterium tuberculosis															

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 55 <212> PRT

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<213> Mycobacterium tuberculosis

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<400> 57

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Thr Leu Ile Arg Asp Ala Val Ser Asp Thr Glu Arg Val Arg Arg Ile
35 40 45

Tyr Tyr Tyr Val Arg Asp Val Pro Tyr Asp Val Leu Ala Ser Phe Arg
50 55 60

20

Tyr Leu Ala Gln Gly His His Arg Ala Ser Asp Val Ile Gly His Gly
65 70 75 80

25

Val Ala Phe Cys Met Gly Lys Ala Ser Ser Phe Val Ala Leu Cys Arg
85 90 95

Ala Ala Gly Val Pro Ala Arg Ile Ala Phe Gln Thr Ile Asp Ala Pro
100 105 110

30

Asp Lys Glu Phe Leu Ser Pro Gln Val Arg Ala Leu Trp Gly Gly Arg
115 120 125

Thr Gly Arg Pro Phe Pro Trp His Ser Leu Gly Glu Ala Tyr Leu Gly
130 135 140

35

Arg Arg Trp Val Lys Leu Asp Ala Thr Ile Asp Ala Pro Thr Ala Ala
145 150 155 160

40

Arg Leu Gly Lys Pro Tyr Arg Gln Glu Phe Asp Gly Ala Thr Pro Ile
165 170 175

Pro Thr Val Glu Gly Thr Ile Leu Arg Glu Asn Gly Ser Tyr Ala Asp
180 185 190

45

Tyr Pro Ser Ala Val Ala Gln Trp Tyr Glu Arg Ile Ala Gln Ser Val
195 200 205

50

Leu Lys Ala Leu Gln Ser Thr Glu Val His Ala Leu Val Ala Ala Asp
210 215 220

Glu Glu Leu Trp Thr Gly Pro Pro Val Glu Leu Ala Asp Ala Thr His
225 230 235 240

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Arg Leu

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 <211> 726
 <212> DNA
 10 <213> Mycobacterium tuberculosis

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 gataccgagc gggtcaggcg gatctactac tacgtgcgcg acgtgccata cgacgtcctc 180
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35 <210> 59
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 <212> PRT
 40 <213> Mycobacterium tuberculosis

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Gly Gln Asn Val Ala Asp Arg Ala Arg Ala Gly Gly Leu Arg Val Ala
 35 40 45

55 Val Val Glu Arg Glu Leu Val Gly Gly Glu Cys Ser Tyr Trp Ala Cys

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25	Thr	Gly	Ser	Arg	Pro 165	Ala	Leu	Pro	Asp	Leu 170	Pro	Gly	Ile	Thr	Glu 175	Ala
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30	Arg	Leu	Ala 195	Ile	Val	Gly	Ala	Gly 200	Gly	Val	Gly	Val	Glu 205	Met	Ala	Thr
35	Ala	Trp 210	Gln	Gly	Leu	Gly	Ala 215	Ser	Val	Thr	Leu	Leu 220	Ala	Arg	Gly	Ser
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40	Gly	Leu	Ala	Asp	Ala 245	Gly	Val	Asp	Val	Arg 250	Val	Gly	Val	Ser	Val 255	Arg
45	Ala	Leu	Gly	Arg 260	Pro	Asn	Pro	Thr	Gly 265	Pro	Val	Val	Leu	Glu 270	Leu	Asp
	Asp	Gly	Thr 275	Glu	Leu	Arg	Val	Asp 280	Glu	Val	Leu	Phe	Ala 285	Thr	Gly	Arg
50	Ala	Pro 290	Arg	Thr	Asp	Asp	Ile 295	Gly	Leu	Glu	Thr	Ile 300	Gly	Leu	Thr	Pro
55	Gly 305	Ser	Trp	Leu	Asp	Val 310	Asp	Asp	Thr	Cys	Arg 315	Val	Arg	Ala	Val	Asp 320

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Asp Gly Trp Leu Tyr Ala Ala Gly Asp Val Asn His Arg Ala Leu Leu
325 330 335

5 Thr His Gln Gly Lys Tyr Gln Ala Arg Ile Ala Gly Thr Ala Ile Gly
340 345 350

Ala Arg Ala Ala Gly Arg Pro Leu Asp Thr Thr Ser Trp Gly Met His
355 360 365

10 Ala Thr Thr Ala Asp His His Ala Val Pro Gln Ala Phe Phe Thr Asp
370 375 380

Pro Glu Ala Ala Ala Val Gly Leu Thr Ala Asp Gln Ala Ala Gln Ala
385 390 395 400

Gly His Arg Ile Lys Ala Ile Asp Val Glu Ile Gly Asp Val Val Met
405 410 415

20 Gly Ala Lys Leu Phe Ala Asp Gly Tyr Thr Gly Arg Ala Arg Met Val
420 425 430

Val Asp Val Asp Arg Gly His Leu Leu Gly Val Thr Met Val Gly Pro
435 440 445

Gly Ala Ala Glu Leu Leu His Ser Ala Thr Val Ala Val Ala Gly Gln
450 455 460

30 Val Pro Ile Asp Arg Leu Trp His Ala Val Pro Cys Phe Pro Thr Ile
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Ser Glu Leu Trp Leu Arg Leu Leu Glu Ser Tyr Arg Asp Ser Phe Tyr
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Leu Leu Val

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<211> 1497
<212> DNA

45 <213> Mycobacterium tuberculosis

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<211> 108

<212> PRT

<213> Mycobacterium tuberculosis

<400> 61

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Ala Ala Ile Ser Glu Val Ala Arg Leu Leu Gly Val Gly Cys Ala Glu
35 40 45

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Thr Val Arg Lys Trp Val Arg Gln Ala Gln Val Asp Ala Gly Ala Arg
 50 55 60
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 Pro Gly Thr Thr Thr Glu Glu Ser Ala Glu Leu Lys Arg Leu Arg Arg
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 35 40 45
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 Leu Gly Ser Thr Gly His Arg Phe Leu Ile Ala Asp Arg Ala Val Asp
 50 55 60

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Pro Pro Gly Asn Gln Leu Met Asn Val His Gly Cys Thr Asp Ala Tyr
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10 Leu Arg Ala Asp Val Leu Pro Leu Pro Gly Gly Leu Thr Val His Leu
100 105 110

Pro Gln Pro Pro Asn Tyr Ala Val Leu Lys Leu His Ala Trp Leu Asp
115 120 125

15 Arg Ser Ala Asp His Asp Tyr Lys Asp Gly Pro Asp Leu Ala Leu Val
130 135 140

20 Val His Trp Tyr Ala Gly Asp Leu Asp Arg Leu Tyr Ala Lys Pro Asp
145 150 155 160

Gln Trp Ala Leu Arg Arg His Asp Phe Asp Leu Arg Thr Ala Ala Ala
165 170 175

25 Ala Leu Leu Gly His Asp Met Arg Ala Ser Val Ser Ala Pro Glu Ala
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195 200 205

Gln His Phe Ala Val Gly Arg Pro Gly
210 215

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<211> 651

40 <212> DNA
<213> Mycobacterium tuberculosis

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55 ctcaaactgc acgcatgget cgatcggtcc gcggaccacg actacaaaga cggcccagat 420

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 <211> 342
 <212> PRT
 15 <213> Mycobacterium tuberculosis

 <400> 65
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 Thr Glu Tyr Gly Val Arg Val Leu Gly Gly Leu Ser Ala Leu Asn Ser
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 35 40 45

 30 Ile Thr Ala Leu Tyr Leu Pro His Leu Ser Ala Thr Ala Ala Leu Gln
 50 55 60
 Ala Trp Asp Thr Ala Gly Ala Gly Ser Pro Leu Leu Val Val Gly Pro
 65 70 75 80
 35 Arg Leu His Pro Ser Ser Ala Glu Thr Leu Arg Ala Arg Gly Leu Trp
 85 90 95

 40 Tyr Ile Asp Gly Ala Gly Asn Ala Tyr Leu Arg His Gln Gly Gly Leu
 100 105 110

 Leu Ile Asp Val Arg Gly Arg Arg Ser Ala Val Ser Ala Gln Pro Gly
 115 120 125
 45 Thr Leu Gly Asp Gly Leu His Ser Asp Gly Pro Arg Asn Pro Phe Thr
 130 135 140

 50 Pro Lys Arg Ala Gln Val Val Cys Val Leu Leu Asp Ala Pro Gln Leu
 145 150 155 160

 Val Asp Ala Pro Leu Arg Ala Ile Ala Ala Ser Ala Gly Val Ser Val
 165 170 175
 55 Gly Met Ala Lys Glu Thr Met Asp Thr Leu Arg Thr Thr Gly Phe Phe

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					180						185					190			
5		Glu	His	Leu	Gly	Ser	Arg	Arg	Arg	Leu	Val	Arg	Thr	Asp	Glu	Leu	Leu		
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		225					230					235					240		
		Leu	Ala	Val	Ala	Val	Ser	Gly	Glu	Gln	Ala	Leu	Pro	Asp	Glu	Ile	Arg		
15						245					250					255			
		Asn	Pro	Glu	Ser	Leu	Met	Leu	Tyr	Val	Asp	Thr	Pro	Ala	Pro	Gly	Leu		
					260					265					270				
20		Pro	Ala	Asp	Leu	Leu	Ile	His	Asn	Arg	Trp	His	Arg	Asp	Pro	His	Gly		
			275						280					285					
		Ser	Ile	Val	Ile	Arg	Lys	Leu	Phe	Trp	Arg	Asn	Leu	Pro	Asp	Glu	Gln		
25			290					295					300						
		Pro	Gly	Leu	Ala	Pro	Thr	Ala	Leu	Ile	Tyr	Ala	Asp	Leu	Leu	Ala	Ser		
		305					310					315					320		
30		Arg	Glu	Pro	Arg	Gln	Val	Glu	Val	Ala	His	Leu	Met	Arg	Arg	Gln	Asp		
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cctgacgagc aaccgggggt ggctcccacg gccttgatct atgcgacct ccttgccctg 960
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20 cgatta 1026

<210> 67

<211> 286

<212> PRT

<213> Mycobacterium tuberculosis

<400> 67

Val Glu Gly Thr Ile Ala Val Pro Gly Gly Arg Val Trp Phe Gln Arg
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Ile Gly Gly Gly Pro Gly Arg Pro Leu Leu Val Val His Gly Gly Pro
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Gly Leu Pro His Asn Tyr Leu Ala Pro Leu Arg Arg Leu Ser Asp Glu
35 40 45

Arg Glu Val Ile Phe Trp Asp Gln Leu Gly Cys Gly Asn Ser Ala Cys
50 55 60

Pro Ser Asp Val Asp Leu Trp Thr Met Asn Arg Ser Val Ala Glu Met
65 70 75 80

Ala Thr Val Ala Glu Ala Leu Ala Leu Thr Arg Phe His Ile Phe Ser
85 90 95

His Ser Trp Gly Gly Met Leu Ala Gln Gln Tyr Val Leu Asp Lys Ala
100 105 110

Pro Asp Ala Val Ser Leu Thr Ile Ala Asn Ser Thr Ala Ser Ile Pro

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		115		120		125	
5	Glu Phe Ser Ala Ser Leu Val Ser Leu Lys Ser Cys Leu Asp Val Ala	130		135		140	
	Thr Arg Ser Ala Ile Asp Arg His Glu Ala Ala Gly Thr Thr His Ser	145		150		155	160
10	Ala Glu Tyr Gln Ala Ala Ile Arg Thr Trp Asn Glu Thr Tyr Leu Cys		165		170		175
15	Arg Thr Arg Pro Trp Pro Arg Glu Leu Thr Glu Ala Phe Ala Asn Met		180		185		190
	Gly Thr Glu Ile Phe Glu Thr Met Phe Gly Pro Ser Asp Phe Arg Ile		195		200		205
20	Val Gly Asn Val Arg Asp Trp Asp Val Val Asp Arg Leu Ala Asp Ile		210		215		220
25	Ala Val Pro Thr Leu Leu Val Val Gly Arg Phe Asp Glu Cys Ser Pro		225		230		235
	Glu His Met Arg Glu Met Gln Gly Arg Ile Ala Gly Ser Arg Leu Glu		245		250		255
30	Phe Phe Glu Ser Ser Ser His Met Pro Phe Ile Glu Glu Pro Ala Arg		260		265		270
35	Phe Asp Arg Val Met Arg Glu Phe Leu Arg Leu His Asp Ile		275		280		285
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	<213> Mycobacterium tuberculosis						
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	cctggtcgtc cgctgcttgt agtgcacggt gggccgggct tgccgcacaa ctacttggcc						120
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	aattccgcat gtccgtcaga cgtagacctt tggacgatga accgctcagt ggccgagatg						240
	gcaaccgtgg cggaagccct tgcccttacc cgttttcaca tcttcagcca ttcgtgggggt						300
55	gggatgctgg cacagcagta cgtgctcgac aaggcgccctg acgcgcgcag tctgaccatc						360

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 5 gccgaatacc aggcgcgat cagaacctgg aacgagactt atctgtgccg caccgcccc 540
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<210> 69

<211> 419

<212> PRT

<213> Mycobacterium tuberculosis

<400> 69

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Pro Tyr Leu Ala Asp Tyr Leu Ala Gly Pro Leu Gly Leu Ala Ala Trp
 35 40 45

Ala Val Gly Leu Val Met Gly Val Arg Asn Phe Ser Gln Gln Gly Met
 50 55 60

Phe Phe Val Gly Gly Thr Leu Ala Asp Arg Phe Gly Tyr Lys Pro Leu
 65 70 75 80

Ile Ile Ala Gly Cys Leu Ile Arg Thr Gly Gly Phe Ala Leu Leu Val
 85 90 95

Val Ala Gln Ser Leu Pro Ser Val Leu Ile Ala Ala Ala Ala Thr Gly
 100 105 110

Phe Ala Gly Ala Leu Phe Asn Pro Ala Val Arg Gly Tyr Leu Ala Ala
 115 120 125

Glu Ala Gly Glu Arg Lys Ile Glu Ala Phe Ala Met Phe Asn Val Phe
 130 135 140

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	Tyr	Gln	Ser	Gly	Ile	Leu	Leu	Gly	Pro	Leu	Val	Gly	Leu	Val	Leu	Leu	145	150	155	160
5	Ala	Leu	Asp	Phe	Arg	Ile	Thr	Val	Leu	Ala	Ala	Ala	Gly	Val	Phe	Gly		165	170	175
	Leu	Leu	Thr	Val	Ala	Gln	Leu	Val	Ala	Leu	Pro	Gln	His	Arg	Ala	Asp	180	185	190	
10	Ser	Glu	Arg	Glu	Lys	Thr	Ser	Ile	Leu	Gln	Asp	Trp	Arg	Val	Val	Val	195	200	205	
15	Arg	Asn	Arg	Pro	Phe	Leu	Thr	Leu	Ala	Ala	Ala	Met	Thr	Gly	Cys	Tyr	210	215	220	
	Ala	Leu	Ser	Phe	Gln	Ile	Tyr	Leu	Ala	Leu	Pro	Met	Gln	Ala	Ser	Ile	225	230	235	240
20	Leu	Met	Pro	Arg	Asn	Gln	Tyr	Leu	Leu	Ile	Ala	Ala	Met	Phe	Ala	Val	245	250	255	
25	Ser	Gly	Leu	Val	Ala	Val	Gly	Gly	Gln	Leu	Arg	Ile	Thr	Arg	Trp	Phe	260	265	270	
	Ala	Val	Arg	Trp	Gly	Ala	Glu	Arg	Ser	Leu	Val	Val	Gly	Ala	Thr	Ile	275	280	285	
30	Leu	Ala	Ala	Ser	Phe	Ile	Pro	Val	Ala	Val	Ile	Pro	Asn	Gly	Gln	Arg	290	295	300	
35	Phe	Gly	Val	Ala	Val	Ala	Val	Met	Ala	Leu	Val	Leu	Ser	Ala	Ser	Leu	305	310	315	320
	Leu	Ala	Val	Ala	Ser	Ala	Ala	Leu	Phe	Pro	Phe	Glu	Met	Arg	Ala	Val	325	330	335	
40	Val	Ala	Leu	Ser	Gly	Asp	Arg	Leu	Val	Ala	Thr	His	Tyr	Gly	Phe	Tyr	340	345	350	
45	Ser	Thr	Ile	Val	Gly	Val	Gly	Val	Leu	Val	Gly	Asn	Leu	Ala	Ile	Gly	355	360	365	
	Ser	Leu	Met	Ser	Ala	Ala	Arg	Arg	Leu	Asn	Thr	Asp	Glu	Ile	Val	Trp	370	375	380	
50	Gly	Gly	Leu	Ile	Leu	Val	Gly	Ile	Val	Ala	Val	Ala	Gly	Leu	Arg	Arg	385	390	395	400
55	Leu	Asp	Thr	Phe	Thr	Ser	Gly	Ser	Gln	Asn	Met	Thr	Gly	Arg	Trp	Ala	405	410	415	

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Ala Pro Arg

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 <210> 70
 <211> 1257
 <212> DNA
 10
 <213> Mycobacterium tuberculosis

<400> 70
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 gggccactgg ggctagccgc gtgggcggtg ggtctggtga tgggcgtgcg caatttctcc 180
 20 cagcaggga tgttcttcgt ggggtggcag ctggccgacg ggttcggcta caagccactg 240
 atcatcgccg gatgtctgat ccgcaccggc gggtttgect tgctggtggt cgcgccagtcg 300
 ctgcccagtg tgctgatcgc cgcggctgcc acgggctttg ccggcgcgct gttcaatccc 360
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 gcgctggatt tccggatcac ggtgctggcc gccgcgggtg tgttcggcct actcaccgtc 540
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 accggatgct atgcgctgtc gttccagatc tatctggctc tgcccatgca ggcgtcgatc 720
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 <210> 71
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 <212> PRT
 55
 <213> Mycobacterium tuberculosis

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 35 40 45
 15 Phe Val Arg Leu Asn Ser Ala Ile Thr Gly Gly Val Gly Trp Trp Tyr
 50 55 60
 20 Ile Leu Val Ala Thr Gly Phe Val Val Phe Ala Leu Tyr Cys Gly Ile
 65 70 75 80
 Ser Arg Ile Gly Thr Ile Arg Leu Gly Arg Asp Asp Glu Leu Pro Glu
 85 90 95
 25 Phe Ser Phe Trp Ala Trp Leu Ala Met Leu Phe Ser Ala Gly Met Gly
 100 105 110
 30 Ile Gly Leu Val Phe Tyr Gly Val Ala Glu Pro Leu Ser His Tyr Leu
 115 120 125
 Arg Pro Pro Arg Ser Arg Gly Val Pro Ala Leu Thr Asp Ala Ala Ala
 130 135 140
 35 Asn Gln Ala Met Ala Leu Thr Val Phe His Trp Gly Leu His Ala Trp
 145 150 155 160
 40 Ala Ile Tyr Val Val Val Gly Leu Gly Met Ala Tyr Met Thr Tyr Arg
 165 170 175
 Arg Gly Arg Pro Leu Ser Val Arg Trp Leu Leu Glu Pro Val Val Gly
 180 185 190
 45 Arg Gly Arg Val Glu Gly Ala Leu Gly His Ala Val Asp Val Ile Ala
 195 200 205
 50 Ile Val Gly Thr Leu Phe Gly Val Ala Thr Ser Leu Gly Phe Gly Ile
 210 215 220
 Thr Gln Ile Ala Ser Gly Leu Glu Tyr Leu Gly Trp Ile Arg Val Asp
 225 230 235 240
 55 Asn Trp Trp Met Val Gly Met Ile Ala Ala Ile Thr Ala Thr Ala Thr

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5	Ala	Ser	Val	Val	Ser	Gly	Val	Ser	Lys	Gly	Leu	Lys	Trp	Leu	Ser	Asn
				260					265					270		
	Ile	Asn	Met	Ala	Leu	Ala	Ala	Ala	Leu	Ala	Leu	Phe	Val	Leu	Leu	Leu
			275					280					285			
10	Gly	Pro	Thr	Leu	Phe	Leu	Leu	Gln	Ser	Trp	Val	Gln	Asn	Leu	Gly	Gly
		290					295					300				
15	Tyr	Val	Gln	Ser	Leu	Pro	Gln	Phe	Met	Leu	Arg	Thr	Ala	Pro	Phe	Ser
	305					310					315					320
	His	Asp	Gly	Trp	Leu	Gly	Asp	Trp	Thr	Ile	Phe	Tyr	Trp	Gly	Trp	Trp
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20	Ile	Ser	Trp	Ala	Pro	Phe	Val	Gly	Met	Phe	Ile	Ala	Arg	Ile	Ser	Arg
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30	Arg	Gln	Arg	Asn	Asn	Gly	Asp	Met	Leu	Val	Asn	Gly	Ala	Val	Asp	Thr
	385					390					395					400
	Asn	Thr	Ser	Leu	Phe	Arg	Leu	Leu	Asp	Gly	Leu	Pro	Ile	Gly	Ala	Ile
35					405					410					415	
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				420					425					430		
40	Ser	Asp	Ser	Gly	Ser	Leu	Val	Ile	Asp	Ile	Leu	Ser	Ala	Gly	Gly	Glu
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	Leu	Asp	Pro	Pro	Lys	Leu	Thr	Arg	Val	Tyr	Trp	Ala	Val	Leu	Glu	Gly
45		450					455					460				
	Val	Ala	Ala	Ala	Val	Leu	Leu	Leu	Ile	Gly	Gly	Ala	Gly	Ser	Leu	Thr
	465					470					475					480
50	Ala	Leu	Arg	Thr	Ala	Ala	Ile	Ala	Thr	Ala	Leu	Pro	Phe	Ser	Ile	Val
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	Met	Val	Val	Ala	Cys	Tyr	Ala	Met	Thr	Lys	Ala	Phe	His	Phe	Asp	Leu
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		530					535					540					
	Ile	Ala	Val	Arg	Asp	Val	Asp	Ser	Gly	Thr	Tyr	Ile	Val	His	Pro	Asp	
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	Thr	Gly	Ala	Leu	Thr	Val	Thr	Ala	Pro	Pro	Asp	Pro	Leu	Asp	Asp	His	
					565					570					575		
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<210> 73

<211> 331

<212> PRT

30

<213> Mycobacterium tuberculosis

35

<400> 73

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Asp Ala Ala Arg Ile Gly Ser Ala Ile Asn Thr Ala Asn Thr Ala Ala
 20 25 30

Ala Ala Thr Thr Gln Val Leu Ala Ala Ala Gln Asp Glu Val Ser Thr
 35 40 45

45

Ala Ile Ala Ala Leu Phe Gly Ser His Gly Gln His Tyr Gln Ala Ile
 50 55 60

50

Ser Ala Gln Val Ala Ala Tyr Gln Gln Arg Phe Val Leu Ala Leu Ser
 65 70 75 80

Gln Ala Gly Ser Thr Tyr Ala Val Ala Glu Ala Ala Ser Ala Thr Pro
 85 90 95

55

Leu Gln Asn Val Leu Asp Ala Ile Asn Ala Pro Val Gln Ser Leu Thr

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5	Gly Arg Pro Leu Ile Gly Asp Gly Ala Asn Gly Ile Asp Gly Thr Gly 115 120 125			
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10	Gly Ser Gly Ala Pro Gly Gln Ala Gly Gly Ala Gly Gly Ala Ala Gly 145 150 155 160			
15	Leu Ile Gly Asn Gly Gly Ala Gly Gly Thr Gly Gly Ala Val Ser Leu 165 170 175			
	Ala Arg Ala Gly Thr Ala Gly Gly Ala Gly Arg Gly Pro Val Gly Gly 180 185 190			
20	Ile Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly Ala Ala Gly Ala 195 200 205			
25	Val Thr Thr Ile Thr His Ala Ser Phe Asn Asp Pro His Gly Val Ala 210 215 220			
	Val Asn Pro Gly Gly Asn Val Tyr Val Thr Asn Phe Gly Ser Gly Thr 225 230 235 240			
30	Val Ser Val Ile Asn Pro Ala Thr Asn Thr Val Thr Gly Ser Pro Ile 245 250 255			
35	Thr Ile Gly Asn Gly Pro Ser Gly Val Ala Val Ser Pro Val Thr Gly 260 265 270			
	Leu Val Phe Val Thr Asn Phe Asp Ser Asn Thr Val Ser Val Ile Asp 275 280 285			
40	Pro Thr Thr Asn Thr Val Thr Gly Ser Pro Ile Thr Val Gly Thr Ala 290 295 300			
45	Pro Thr Gly Val Ala Val Asn Pro Val Thr Gly Glu Val Tyr Val Thr 305 310 315 320			
	Asn Phe Ala Gly Asp Thr Val Ser Val Ile Ser 325 330			
50	<210> 74			
	<211> 993			
	<212> DNA			
55	<213> Mycobacterium tuberculosis			

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5 <400> 74
 atgtcgtttg tcaacgtggc cccacagtta gtgtccacag ccgcggccga tgcagcgcgg 60
 atcggctcgg cgatcaacac cgccaacacc gcggcgccgg cgaccacca ggtgttgccc 120
 gccgccaag acgaggtgtc aacggcgatc gccgcgtgt tcggcagcca cggccagcac 180
 10 tatcaagcga tcagcgcgca ggtcgcggcc tatcagcaac ggttcgtgct ggccttaagc 240
 caagctggca gcacctacgc ggtcgcgcaa gcggccagcg caacaccgct gcagaacgtg 300
 ctcgatgcga tcaacgcacc cgttcagtcg ctgaccgggc gccattgat cggcgacggc 360
 15 gcgaacggga tcgacgggac cgggcaagcc ggcggtaacg gcgggtggct gtggggcaac 420
 ggcggaacg gcgggtcggg ggcacccgga caggccggcg gcgccggcg ggcgccggg 480
 ttgatcggca acggtggggc cggcgccacc ggcgcgccgg tcagcctcgc ccgcgcggc 540
 20 acggccggcg gtgcggccg cgcccggtc ggcggtatcg gcggtgcggg tggggtcggc 600
 ggtgccggtg gggccgcccg cgccgtcacc accatcacc acgccagctt caacgatccg 660
 cacggggtg cggtcaaccc gggcggaac gtctacgtca ccaatttcgg cagcggcacg 720
 25 gtgtcgtga tcaacccgc caccaacacc gtcaccggt ccccatcac catcggaac 780
 ggtccaagcg ggggtggcgt cagccccgtc accggcctgg tcttcgtgac caacttcgac 840
 agcaacacgg tgtcgtgat cgaccgacc accaacaccg tcaccggctc ccccatcacc 900
 30 gtcggcaccg ctccgaccg ggtggcggtc aacccgtca ccggcgaggt ttatgtcacc 960
 aacttcgccg gcgacacggt gtcggtaatc agc 993

35 <210> 75
 <211> 251
 <212> PRT
 <213> Mycobacterium tuberculosis

40 <400> 75
 Met Arg Ala Asp Val Thr Ala Glu His Leu Thr Gln Val Val Arg Asp
 1 5 10 15
 45 Ile Ala Val Ile Asp Ile Asp Asp Gly Val Ala Phe Asn Leu Asp Thr
 20 25 30
 50 Ser Ser Val Gln Glu Ile Arg Glu Arg Ala Asp Tyr Pro Gly Leu Arg
 35 40 45
 Val Arg Val Ala Met Ser Val Gly Pro Trp Gln Gly Ile Ala Ala Trp
 50 55 60
 55 Asp Val Ser Thr Gly Glu Pro Ile Ala Pro Trp Pro Thr Arg Val Thr

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	65		70		75		80	
5	Ile Asp Arg Ile	Leu Gly Glu Pro Ile Thr Leu Leu Gly Tyr Ala Pro	85	90	95			
	Glu Thr Ile Ile	Ala Glu Lys Gly Val Thr Ile Leu Glu Arg Gly Ile	100	105	110			
10	Thr Ser Thr Arg	Trp Arg Asp Tyr Val Asp Ile Val Gln Leu Asp Arg	115	120	125			
15	Arg Gly Ile Asp	Asp Asp Glu Leu Leu Arg Ser Ala Arg Ala Val Ala	130	135	140			
	Gln Tyr Arg Gly	Ala Thr Leu Glu Pro Val Ala Pro His Leu Ala Gly	145	150	155			160
20	Tyr Gly Ala Val	Ala Gln Ala Lys Trp Ala Thr Glu His Gly Arg Cys	165	170	175			
25	Gln His Cys Trp	Arg His Trp Lys Pro Ala His Val Gly Arg Arg Asn	180	185	190			
	Met Asp Leu Leu	Asp Ala Lys Gln Val Ser Glu Met Ile Gly Val Pro	195	200	205			
30	Val Gly Thr Leu	Arg His Trp Arg His Ser Asp Ile Gly Pro Ala Ser	210	215	220			
35	Phe Thr Leu Gly	Arg Arg Val Val Tyr Arg Arg Asp Glu Val Ser Arg	225	230	235			240
	Trp Ile Ser Lys	Arg Glu Ser Ala Thr Arg Arg	245	250				
40	<210>	76						
	<211>	753						
	<212>	DNA						
45	<213>	Mycobacterium tuberculosis						
	<400>	76						
50	atgcgcgcgcg	acgtcaccgc cgagcatctc acccaggtgg ttcgcgacat cgccgtgatc						60
	gacatcgacg	acgggggtggc gttcaacctc gacacgagca gcgtgcagga aattcgcgag						120
	cgggcgcgact	accgcggcct gcgcgtgcga gtcgctatgt cggtcggacc gtggcagggc						180
	atcgcggcct	gggatgtgtc caccggcgaa ccgatcgcgc cgtggcccac acgggtgacc						240
55	atcgaccgga	tctcggcgga gccgatcaca ctctgggct acgcgccga gaccatcatc						300

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gccgagaagg gagtgaccat cctcgaacgc ggcacaccca gcacccgctg gcgggactac 360
 5 gtcgacatcg tccaactcga ccgccggggc atcgacgacg acgagctgct ccgctcggcc 420
 agggcagtcg cacaataccg cggcgccact ctccaacccg tcgcgcctca cctggccggt 480
 tatggcgtag tcgcgcaagc gaaatgggag accgaacacg gacgctgcca gcactgttgg 540
 10 agacattgga aaccagccca tgtcgggagg agaaacatgg atctgctgga cgcaaaacaa 600
 gtttcggaga tgatcggcgt tcccgtcggc actctacggc actggcggca ctcgacatc 660
 ggaccggcga gcttcacctt gggacggcgc gtcgtgtacc ggcgcgacga ggtgtcgcgc 720
 15 tggatctcaa agcgggagag cgcaactcga cgt 753

<210> 77

<211> 254

20 <212> PRT

<213> Mycobacterium tuberculosis

25 <400> 77

Met Ser Val Asp Tyr Pro Gln Met Ala Ala Thr Arg Gly Arg Ile Glu
1 5 10 15

30 Pro Ala Pro Arg Arg Val Arg Gly Tyr Leu Gly His Val Leu Val Phe
20 25 30

Asp Thr Ser Ala Ala Arg Tyr Val Trp Glu Val Pro Tyr Tyr Pro Gln
35 35 40 45

Tyr Tyr Ile Pro Leu Ala Asp Val Arg Met Glu Phe Leu Arg Asp Glu
50 55 60

40 Asn His Pro Gln Arg Val Gln Leu Gly Pro Ser Arg Leu His Ser Leu
65 70 75 80

Val Ser Ala Gly Gln Thr His Arg Ser Ala Ala Arg Val Phe Asp Val
85 90 95

45 Asp Gly Asp Ser Pro Val Ala Gly Thr Val Arg Phe Asn Trp Asp Pro
100 105 110

50 Leu Arg Trp Phe Glu Glu Asp Glu Pro Ile Tyr Gly His Pro Arg Asn
115 120 125

Pro Tyr Gln Arg Ala Asp Ala Leu Arg Ser His Arg His Val Arg Val
130 135 140

55 Glu Leu Asp Gly Ile Val Leu Ala Asp Thr Arg Ser Pro Val Leu Leu

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	145		150		155		160									
5	Phe	Glu	Thr	Gly	Ile	Pro	Thr	Arg	Tyr	Tyr	Ile	Asp	Pro	Ala	Asp	Ile
					165					170					175	
	Ala	Phe	Glu	His	Leu	Glu	Pro	Thr	Ser	Thr	Gln	Thr	Leu	Cys	Pro	Tyr
				180					185					190		
10	Lys	Gly	Thr	Thr	Ser	Gly	Tyr	Trp	Ser	Val	Arg	Val	Gly	Asp	Ala	Val
			195					200					205			
	His	Arg	Asp	Leu	Ala	Trp	Thr	Tyr	His	Tyr	Pro	Leu	Pro	Ala	Val	Ala
15		210					215					220				
	Pro	Ile	Ala	Gly	Leu	Val	Ala	Phe	Tyr	Asn	Glu	Lys	Val	Asp	Leu	Thr
	225					230					235				240	
20	Val	Asp	Gly	Val	Ala	Leu	Pro	Arg	Pro	His	Thr	Gln	Phe	Ser		
					245					250						
25	<210>	78														
	<211>	762														
	<212>	DNA														
	<213>	Mycobacterium tuberculosis														
30	<400>	78														
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	tgggagggttc	cctactaccc	gcagtactac	atcccgcctgg	cggatgtccg	catggagttc										180
	ctgcgcgacg	agaaccaccc	gcagcgagtg	cagctggggtc	cgtcgcgggt	gcactccttg										240
40	gtaagcgccg	gtcagaccca	ccgatcggcg	gcgcgggtat	tcgatgtcga	cggcgacagc										300
	ccggtggcgg	gcaccgtgcg	tttcaactgg	gatccgctgc	ggtgggttcga	ggaggacgag										360
	ccgatctacg	gccatccgcg	caatccctat	cagcggggccg	atgcgctgcg	ctcgcaccga										420
45	cacgtccgtg	tcgagctgga	cggcattgtg	ctcgtcgaca	cccgatcgcc	cgttctgcta										480
	ttcgaaactg	ggatacccac	aaggtattac	atcgatccgg	ccgacatcgc	tttcgagcat										540
	ctggagccca	cctcgacgca	gacgttgtgt	ccgtacaagg	ggacgacgtc	gggctatttg										600
50	tctgtgcgog	tcggcgacgc	cgtgcaccgc	gacctggcct	ggacgtatca	ctatccactg										660
	cccgcogttg	ccccgatcgc	cggcctgggtg	gcgtttttaca	acgagaaggt	cgacctcacc										720
	gtcgacggcg	tcgcctgcc	gcggcgcac	actcagttca	gc											762
55	<210>	79														

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<211> 120

<212> PRT

5 <213> Mycobacterium tuberculosis

<400> 79

10 Ser Phe Ala Gly Ala Glu Ala Ala Asn Ala Ser Gln Leu Gln Ser Ile
1 5 10 15

Ala Arg Gln Val Arg Gly Ala Val Asn Ala Val Ala Gly Gln Val Thr
15 20 25 30

Gly Asn Gly Gly Ser Gly Asn Ser Gly Thr Ser Ala Ala Ala Ala Asn
35 40 45

20 Pro Asn Ser Asp Asn Thr Ala Ser Ile Ala Asp Arg Gly Thr Ser Ala
50 55 60

Ile Met Thr Thr Ala Ser Ala Thr Ala Ser Ser Thr Gly Val Asp Gly
65 70 75 80

25 Gly Ile Ala Ala Thr Tyr Ala Val Ala Ser Gln Trp Asp Gly Gly Tyr
85 90 95

30 Val Ala Asn Tyr Thr Ile Thr Gln Phe Gly Arg Asp Phe Asp Asp Arg
100 105 110

Leu Ala Val Ala Ile His Phe Ala
115 120

35 <210> 80
<211> 360

40 <212> DNA

<213> Mycobacterium tuberculosis

<400> 80

45 tctttcgccg gcgccgaggc cgccaatgcg tcacagctgc agagcatcgc gcggcaggtg 60

cggggcgcgc tcaacgccgt cgccggtcag gtgacgggca atggcggtc cggaacagc 120

ggcacttcgg ctgcggcggc caaccgaat tccgacaaca cagcgagcat cgccgatagg 180

50 ggcacaagcg ccatcatgac cacggcaagc gcgaccgcgt cttccacggg cgtcgatggc 240

ggaatagcgg cgacgtatgc ggtgcctcgc caatgggatg gtggctacgt ggccaattac 300

acgatcacco aattcgggcg cgaattcgat gaccgattgg cggttgcaat tcaatttgcc 360

55 <210> 81

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<211> 470
 <212> PRT
 <213> Mycobacterium tuberculosis

<400> 81

Val Ala Thr Val Ala Phe Val Ala Thr Ala Ser Ile Val Ile Thr Pro
 1 5 10 15

Ala Ala Ile Val Leu Leu Gly Pro Arg Leu Asp Ala Leu Asp Val Arg
 20 25 30

Arg Leu Val Arg Arg Leu Leu Gly Arg Pro Asp Pro Val His Lys Pro
 35 40 45

Val Lys Gln Leu Phe Trp Tyr Arg Ser Ser Lys Phe Val Met Arg Arg
 50 55 60

Trp Leu Pro Val Gly Thr Ala Val Val Ala Leu Leu Val Leu Leu Gly
 65 70 75 80

Leu Pro Phe Leu Ser Val Lys Trp Gly Phe Pro Asp Asp Arg Val Leu
 85 90 95

Pro Arg Ser Ala Ser Ala Arg Gln Val Gly Asp Ile Leu Arg Asp Asp
 100 105 110

Phe Gly His Asp Pro Ala Thr Gln Ile Pro Ile Val Val Pro Asp Ala
 115 120 125

Arg Gly Leu Gly Pro Val Glu Leu Asp Ser Tyr Ala Ala Glu Leu Ser
 130 135 140

Arg Val Pro Asp Val Ser Ala Val Ala Ala Pro Thr Gly Thr Phe Val
 145 150 155 160

Asp Gly Ser Trp Val Gly Thr Pro Arg Gly Ala Thr Gly Leu Ala Glu
 165 170 175

Gly Ser Ala Phe Leu Thr Val Ser Ser Thr Ala Pro Leu Phe Ser Arg
 180 185 190

Ala Ser Asp Ile Gln Leu Lys Arg Leu His Gln Val Ala Gly Pro Ala
 195 200 205

Gly Arg Ser Val Val Met Ala Gly Val Ala Gln Val Asn Arg Asp Ser
 210 215 220

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Val Asp Ala Val Thr Asp Arg Leu Pro Met Val Leu Gly Leu Ile Ala
225 230 235 240

5 Ala Ile Thr Tyr Val Leu Leu Phe Leu Leu Thr Gly Ser Val Val Leu
245 250 255

Pro Ala Lys Ala Leu Val Cys Asn Val Leu Ser Leu Thr Ala Ala Phe
260 265 270

10 Gly Ala Leu Val Trp Ile Phe Gln Glu Gly His Phe Gly Ala Leu Gly
275 280 285

15 Thr Thr Pro Ser Gly Thr Leu Val Ala Asn Met Pro Val Leu Leu Phe
290 295 300

Cys Ile Ala Phe Gly Leu Ser Met Asp Tyr Glu Val Phe Leu Val Ser
305 310 315 320

20 Arg Ile Arg Glu Tyr Trp Leu Glu Ser Gly Ala Ala Arg Pro Ala Arg
325 330 335

25 Arg Ser Val Ala Glu Val His Ala Ala Asn Asp Glu Ser Val Ala Leu
340 345 350

Gly Val Ala Arg Thr Gly Arg Val Ile Thr Ala Ala Ala Leu Val Met
355 360 365

30 Ser Met Ser Phe Ala Ala Leu Ile Ala Ala His Val Ser Phe Met Arg
370 375 380

35 Met Phe Gly Leu Gly Leu Thr Leu Ala Val Ala Ala Asp Ala Thr Leu
385 390 395 400

Val Arg Met Val Val Val Pro Ala Phe Met His Val Thr Gly Arg Trp
405 410 415

40 Asn Trp Trp Ala Pro Arg Pro Leu Ala Trp Leu His Glu Arg Phe Gly
420 425 430

45 Val Ser Glu Ala Ala Glu Pro Val Ser Arg Arg Arg Ser His Ala Gly
435 440 445

Gly Leu Gly Lys Ile Ala Gly Arg Ser Asp Gly Gln Thr Ile Pro Ala
450 455 460

50 Ser Leu Thr Arg Asn Gly
465 470

55 <210> 82

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<211> 1410

<212> DNA

5 <213> Mycobacterium tuberculosis

<400> 82

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	cggcccgatc cgggtgcacaa accgggtcaag caactgttct ggtaccggtc gagcaagttc	180
15	gtgatgcgcc gttggctgcc ggctcggtacg gctgttgctcg cgtgctggt gctgctcggg	240
	ctgccgttct tgtcggtgaa gtgggggttc ccgacgacc ggggtgttgc gcggtcggcg	300
	tcggcccgtc aagtcggcga tatcttgccg gatgactttg gccacgatcc tgcgacgcag	360
20	atacccatcg tcgtcccgga cgtcgtggt ctcgcccccg tcgaacttga cagctacgca	420
	gccgagttgt cccgggtgcc cgacgtatcc gcggtagccg ccccgacggg caogttcgta	480
	gacggcagct ggggtgggaa gccgcgcggg gccacccggg tggctgaggg cagcgcgttc	540
25	ctgacgggtga gcagcacggc gccgctgttt tcgcgagcct ccgatatcca gctcaagcgg	600
	ttgcaccagg tggcagggcc ggccgggtcga tccgtcgtga tggccgggtg cgcgcaggtc	660
	aaccgcgaca gtgtcgacgc ggtgaccgat cggcttcoga tgggtgctagg gctaattgcc	720
30	gcgatacct acgtactgtt gttcctgctc accggcagcg tgggtgctgc ggcgaaagcg	780
	ttggtttgta atgtgttata gctgaccgcg gcgtttggcg cgttggtgtg gatcttccag	840
	gaaggccatt tcggtgccct gggaacgact ccgagcggga cgttggtggc gaatatgccg	900
35	gtcctactgt tttgcatcgc attcggtttg tccatggact acgaggtgtt tctggtctcc	960
	aggattcggg agtactggtt ggaatccgga gccgcgcgac ccgcgcgaag aagcgtcgca	1020
	gaggtgcacg ccgccaacga cgagagcgtc gcgctcggcg tggcccgcac cggtcgggtg	1080
40	atcacccgcg cagcgttggt gatgtccatg tcgttcgcgc cgttgatcgc tgcgcacgtg	1140
	tcgttcatgc ggatgttcg cctcggcctg acttttagccg tggctgcaga cgccacactg	1200
	gtgcggatgg tcgtggtccc agcattcatg catgtgacgg gccgctggaa ttggtgggca	1260
45	ccgagacccc tggcgtggct gcatgagcgg ttcggtgtca gcgaggcagc agagccggtt	1320
	tcgaggagac gttcccacgc cgggtgggtt ggcaagattg ccggacgaag cgacggtcag	1380
	acgatccctg cctcgtgac gcgcaatggt	1410

50 <210> 83

<211> 216

<212> PRT

55 <213> Mycobacterium tuberculosis

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<400> 83

5 Met Thr Ser Gly Ala Ala Ala Ser Ala Ser Arg Val Asp His Pro Leu
1 5 10 15

Phe Ala Arg Ile Trp Pro Val Val Ala Ala His Glu Ala Glu Ala Ile
20 25 30

Arg Ala Leu Arg Arg Glu Asn Leu Ala Gly Leu Ser Gly Arg Val Leu
35 40 45

15 Glu Val Gly Ala Gly Val Gly Thr Asn Phe Ala Tyr Tyr Pro Val Ala
50 55 60

Val Glu Gln Val Ile Ala Met Glu Pro Glu Pro Arg Leu Ala Ala Lys
65 70 75 80

Ala Arg Ile Ala Ala Ala Asp Ala Pro Val Pro Ile Val Val Thr Asp
85 90 95

25 Lys Thr Val Glu Glu Phe Arg Asp Thr Glu Thr Phe Asp Ala Val Val
100 105 110

Cys Ser Leu Val Leu Cys Ser Val Ser Asp Pro Gly Ala Val Leu Ala
115 120 125

His Leu Arg Ser Leu Leu Arg Arg Gly Gly Glu Leu Arg Tyr Leu Glu
130 135 140

35 His Val Ala Ser Ala Gly Ala Arg Gly Arg Val Gln Arg Phe Val Asp
145 150 155 160

Ala Thr Phe Trp Pro Arg Leu Ala Gly Asn Cys His Thr His Arg His
165 170 175

Thr Glu Arg Ala Ile Leu Asp Ala Gly Phe Val Val Asp Ser Ser Arg
180 185 190

45 Arg Glu Trp Ala Phe Pro Ala Trp Val Pro Leu Pro Val Ser Glu Leu
195 200 205

Ala Leu Gly Arg Ala His Arg Thr
210 215

50 <210> 84

<211> 648

<212> DNA

55 <213> Mycobacterium tuberculosis

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5 <400> 84
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 gccgggtttgt cggggcggggt gttggaagtc ggggccggcg tcgggacgaa ctttgccctac 180
 10 taccgcggtgg ccgtcgaaca ggtcatcgcc atggagcccg agccgcggct tgctgccaaag 240
 gcccgcatcg cggccgctga cgcaccgctt ccgatatgctg tgacggacaa gacggtcgag 300
 gagttccgcg acaccgagac gtttgacgcg gtggtttgct cgctgggtgct gtgctcgggtg 360
 15 agcgaccgag gcgcgggtgct ggccgacctg cgttcgctac tacggcgagg cggggagctg 420
 cgctatctcg agcatgtggc cagcgccggc gctcgggggc ggggtgcagcg gttcgtcgac 480
 gcgacatttt ggcccaggct ggccgggcaac tgcacacgc atcgccatac cgaacgcgcg 540
 20 atcctcgacg ccgattcgt ggtggacagc tcccgccggg agtgggcatt tccgcctgg 600
 gtgccgctac cgggtgtcaga gttggctctg ggccgcgcgc accggacc 648

25 <210> 85
 <211> 108
 <212> PRT

30 <213> Mycobacterium tuberculosis

<400> 85
 35 Met Ser Gly Gly Ser Ser Arg Arg Tyr Pro Pro Glu Leu Arg Glu Arg
 1 5 10 15
 Ala Val Arg Met Val Ala Glu Ile Arg Gly Gln His Asp Ser Glu Trp
 20 25 30
 40 Ala Ala Ile Ser Glu Val Ala Arg Leu Leu Gly Val Gly Cys Ala Glu
 35 40 45
 Thr Val Arg Lys Trp Val Arg Gln Ala Gln Val Asp Ala Gly Ala Arg
 50 55 60
 45 Pro Gly Thr Thr Thr Glu Glu Ser Ala Glu Leu Lys Arg Leu Arg Arg
 65 70 75 80
 50 Asp Asn Ala Glu Leu Arg Arg Ala Asn Ala Ile Leu Lys Thr Ala Ser
 85 90 95
 Ala Phe Phe Ala Ala Glu Leu Asp Arg Pro Ala Arg
 100 105
 55

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<210> 86
 <211> 324
 5 <212> DNA
 <213> Mycobacterium tuberculosis

10 <400> 86
 atgtcaggtg gttcatcgag gaggtacccg ccgagagctgc gtgagcgggc ggtgcggatg 60
 gtcgcagaga tccgcgggtca gcacgattcg gagtgggcag cgatcagtga ggtcgcccg 120
 ctacttggtg ttggctgctc ggagacgggtg cgtaagtggg tgcgccaggc gcaggtcgat 180
 15 gccggcgcac ggcccgggac caccgaccgaa gaatccgctg agctgaagcg cttgcggcgg 240
 gacaacgccg aattgcgaag ggcgaacgcg attttaaaga ccgcgtcggc tttcttcgcg 300
 gccgagctcg accggccagc acgc 324
 20

<210> 87
 <211> 489
 25 <212> PRT
 <213> Mycobacterium tuberculosis

30 <400> 87
 Val Thr Asn Asp Leu Pro Asp Val Arg Glu Arg Asp Gly Gly Pro Arg
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 35 Pro Ala Pro Pro Ala Gly Gly Pro Arg Leu Ser Asp Val Trp Val Tyr
 20 25 30
 40 Asn Gly Arg Ala Tyr Asp Leu Ser Glu Trp Ile Ser Lys His Pro Gly
 35 40 45
 Gly Ala Phe Phe Ile Gly Arg Thr Lys Asn Arg Asp Ile Thr Ala Ile
 50 55 60
 45 Val Lys Ser Tyr His Arg Asp Pro Ala Ile Val Glu Arg Ile Leu Gln
 65 70 75 80
 50 Arg Arg Tyr Ala Leu Gly Arg Asp Ala Thr Pro Arg Asp Ile His Pro
 85 90 95
 Lys His Asn Ala Pro Ala Phe Leu Phe Lys Asp Asp Phe Asn Ser Trp
 100 105 110
 55 Arg Asp Thr Pro Lys Tyr Arg Phe Asp Asp Pro Asn Asp Leu Leu His
 115 120 125

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5 Arg Val Lys Ala Arg Leu Ala Glu Pro Ala Leu Ala Ala Arg Ile Lys
 130 135 140
 Arg Met Asp Thr Leu Phe Asn Ala Ile Val Ala Val Leu Ala Val Gly
 145 150 155 160
 10 Tyr Phe Ala Val Gln Gly Val Arg Leu Val Glu Pro Ser Trp Met Pro
 165 170 175
 Leu Trp Ala Phe Val Ile Ala Met Val Leu Leu Arg Ser Ser Leu Ala
 15 180 185 190
 Gly Phe Gly His Tyr Ala Leu His Arg Ala Gln Arg Gly Leu Asn Arg
 195 200 205
 20 Val Phe Asn Asn Ala Phe Asp Leu Asn Tyr Val Ala Leu Ser Leu Val
 210 215 220
 Thr Ala Asp Gly His Thr Leu Leu His His Pro Tyr Thr Gln Ser Glu
 25 225 230 235 240
 Val Asp Ile Lys Lys Asn Val Phe Thr Met Met Met Arg Leu Pro Trp
 245 250 255
 30 Leu Tyr Arg Val Pro Val His Thr Ile His Lys Phe Gly His Met Leu
 260 265 270
 Ser Gly Met Ala Ile Arg Ile Val Asp Val Phe Arg Ile Thr Arg Lys
 35 275 280 285
 Val Gly Val Glu Glu Ser Tyr Gly Ser Trp Arg Ala Ala Leu Pro His
 290 295 300
 40 Phe Leu Gly Ser Ala Gly Val Arg Leu Leu Leu Val Ser Glu Leu Val
 305 310 315 320
 Val Phe Ala Ile Ala Gly Asp Phe Trp Pro Trp Ala Leu Gln Phe Val
 45 325 330 335
 Ala Thr Leu Trp Val Ser Thr Phe Leu Val Val Ala Ser His Glu Phe
 340 345 350
 50 Glu Asp Asp Thr Gln Gly Gly Ala Val Asn Gly Glu Asp Trp Gly Ile
 355 360 365
 Asp Gln Leu Glu His Ala Asn Asp Leu Thr Val Ile Gly Asn Arg Tyr
 55 370 375 380

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Val Asp Cys Phe Leu Ser Ala Gly Leu Ser Ser His Arg Val His His
385 390 395 400

5 Val Leu Pro Phe Gln Arg Ser Gly Phe Ala Asn Ile Val Thr Glu Asp
405 410 415

10 Val Leu Arg Glu Glu Ala Ala Lys Phe Gly Val Glu Trp Leu Pro Ala
420 425 430

Lys Gly Phe Ile Thr Asp Arg Leu Pro Arg Leu Cys Arg Lys Tyr Leu
435 440 445

15 Leu Thr Pro Ser Arg Gln Ala Lys Glu Arg His Trp Gly Phe Val Arg
450 455 460

Glu His Cys Ser Pro Ala Ala Leu Lys Ala Ser Ala Ser Tyr Val Val
465 470 475 480

20 Ala Gly Phe Val Gly Ile Gly Ser Val
485

25 <210> 88

<211> 1467

<212> DNA

30 <213> Mycobacterium tuberculosis

<400> 88
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35 gctggcgggc cacgcttgct agacgtgtgg gtttacaacg ggcgggcgta cgacctgagt 120

gagtggattt ccaagcatcc cggcggcgcc ttcttcattg ggcggaccaa gaaccgcgac 180

atcacgcgaa tcgtcaagtc ctaccatcgt gatccggcga ttgtcgagcg aatcctgcag 240

40 cggaggtacg cgttggggccg cgacgcaacc ctaggggaca tccaccccaa gcacaatgca 300

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gacgacccca acgatctgct gcaccgggtc aaagcgcggc tagccgagcc agcgctggcc 420

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<211> 393

<212> PRT

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<213> Mycobacterium tuberculosis

<400> 89

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Gln Ser Asp Ala Ala Asp Arg Val Ala Glu Leu Phe Leu Asp Pro Gly
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Gln Arg Glu Arg Ile Pro Arg Val Tyr Gln Lys Ser Arg Ile Thr Thr
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Arg Arg Met Ala Val Asp Pro Leu Asp Ala Lys Phe Asp Val Phe Arg
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Arg Glu Pro Ala Thr Ile Arg Asp Arg Met His Leu Phe Tyr Glu His
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Ala Val Pro Leu Ala Val Asp Val Ser Lys Arg Ala Leu Ala Gly Leu
 115 120 125

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<212> DNA

<213> Mycobacterium tuberculosis

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<212> PRT

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<213> Mycobacterium tuberculosis

<400> 91

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<212> PRT

<213> Mycobacterium tuberculosis

<400> 93

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15

Ala Glu Ile Arg Gly Leu Asp Tyr Asp Ala Arg Met Ala Asp Leu Trp
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Arg Ala Ser Ile Thr Glu Asn Phe Val Thr Ala Val His Tyr Leu Asp
50 55 60

Arg Asp Thr Pro Gln Ser Leu Val Glu Ala Pro Ala Ala Ala Leu Ala
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25

Tyr Ala Arg Ala Ala Ala Gln Arg Asp Ile Pro Leu Ser Gly Leu Val
85 90 95

Arg Ala His Arg Leu Gly His Ala Arg Phe Leu Glu Val Ala Met Gln
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Tyr Val Ser Leu Leu Glu Pro Ala Asp Arg Val Ser Thr Ile Ile Glu
115 120 125

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Leu Val Asn Arg Ser Ala Arg Leu Val Asp Leu Val Ala Asp Gln Leu
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Gly Leu Gln Gln Gln Trp Val Ser Glu Leu Leu Ala Asp Thr Pro Val
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Asp Val Pro Arg Ala Glu Arg Ala Leu Gly Tyr Arg Leu Asp Gly Val
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195 200 205

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Val Val Ala Gln Phe Asp Gln Val Arg Cys Leu Leu Ala Gly Glu Leu
210 215 220

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Gly Pro Glu Leu Gly Pro Val Ala Asn Ser Leu Met Val Pro Thr Asp
225 230 235 240

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 15 Ala Arg Pro Gly Gly Arg Val Met Phe Tyr Asp Asp Val Ala Pro Val
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40 <213> Mycobacterium tuberculosis

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55 Asp Pro Glu Ile Val His Ala Asp Leu Val Ile Leu Arg Ala Thr Arg
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 5 Ala Asn Leu Leu Asn Ser Arg Pro Val Val Ala Trp Asn Val Glu Arg
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<213> Mycobacterium tuberculosis

<400> 101

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Thr Gly Val Ala Tyr Gly Pro Asp Ser Asp Glu Gln Ile Asn Thr Val
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Ala Val Gly Arg Glu Lys His Arg Cys Ser Leu Asp Pro Leu Gly Gly
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Ala Ile Val Ser Lys Val Tyr Pro Met Arg Ser His Thr Val Ser Ala
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Glu Gly Gly Ala Ala Ala Val Thr Gly Asp Asp Asp Ser Leu Asp Glu
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His Ala His Asp Thr Val Ser Gly Gly Asp Trp Leu Cys Asp Gln Asp
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Ala Val Glu Ala Phe Val Ala Glu Ala Pro Lys Glu Leu Val Gln Leu
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Glu His Trp Gly Cys Pro Trp Ser Arg Lys Pro Asp Gly Arg Val Ala
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 15 Glu Ala Leu Arg Leu Glu Arg Glu Leu Ser Arg His Gly Gln Gly Gly
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 Glu Arg Ile Ala Asp Ile Arg Ala Asp Met Gln Ala Thr Leu Glu Ser
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 <211> 1749
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 50 <213> Mycobacterium tuberculosis
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15 Asp Tyr Leu Gly Leu Ser Arg His Pro Ala Val Ile Asp Gly Gly Val
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Gln Ala Leu Arg Ile Trp Gly Ala Gly Ala Thr Gly Ser Arg Leu Val
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25 Phe Val Gly Ala Ala Ala Gly Leu Leu Phe Ser Ser Gly Tyr Thr Ala
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Asn Leu Gly Ala Val Val Gly Leu Ser Gly Pro Gly Ser Leu Leu Val
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Ala Ala Leu Arg Ser Arg Asp Glu Gln Arg Ala Val Val Val Thr Asp
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Gly Leu Gly Val Arg Gly Gly Gly Arg Gly Leu Leu Tyr Glu Leu Gly
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55 Leu Gly Ser Gln Gly Gly Val Val Leu Gly Pro Thr Pro Val Arg Ala
245 250 255

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<212> PRT

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<213> Mycobacterium tuberculosis

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<400> 109

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40

Leu Val Arg Leu Gln Ala Ala Leu Asn Arg His Glu His Thr Gly Glu
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Leu Glu Ala Phe Leu Arg Ile Ser Val Glu His Ala Asp Ala Ala Gly
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Gly Asp Glu Cys Gly Pro Ala Ile Leu Ala Gly Arg Ser Gly Pro Glu
85 90 95

50

Gln Ala Ala Ile Asn Arg Gln Leu Gly Leu Ala Gly Asp Asp Glu Pro
100 105 110

55

Asp Gly Asp Asp Thr Pro Pro Trp Ser Arg Met Ile Gly Leu Gly Gly
115 120 125

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130 135

5 <210> 110
<211> 408
<212> DNA
10 <213> Mycobacterium tuberculosis

<400> 110
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gcgcccttgg aggtgaggga agcgcctcgtt cggtgcagg ccgcgttgaa tcggcacgag 180
20 cacaccggcg agctcgaagc gttcctgctg atcagcgctg agcacgccga cgcgcgcggc 240
ggcgacgagt gcggcccggc gatcctggcc ggccgctcgc ggccggaaca agccgccatc 300
aaccggcaac tcggactcgc cggcgacgac gagcccgacg gcgacgacac cccgcctgg 360
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30 <212> PRT
<213> Mycobacterium tuberculosis

35 <400> 111
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20 25 30
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35 40 45
45 Gly His Lys Leu Lys Leu Leu Val Ser His Asp Ala Arg Thr Arg Tyr
50 55 60
50 Pro Val Gly Arg Ala Val Glu Leu Arg Glu Glu Pro His Gly Leu Phe
65 70 75 80
Gly Ala Phe Glu Ile Ala Asp Thr Pro Asp Gly Asp Glu Ala Leu Ala
85 90 95
55

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Asn Val Lys Ala Gly Val Val Asp Ser Phe Ser Val Gly Phe Arg Pro
100 105 110

5 Ile Arg Asp Arg Arg Glu Gly Asp Val Leu Val Arg Val Glu Ala Ala
115 120 125

Leu Leu Glu Val Ser Leu Thr Gly Val Pro Ala Tyr Ser Gly Ala Gln
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Ile Ala Gly Val Arg Ala Glu Ser Leu Thr Val Val Ser Arg Ser Thr
145 150 155 160

15 Ala Glu Ala Trp Leu Ser Leu Leu Asp Trp
165 170

<210> 112

20 <211> 510

<212> DNA

<213> Mycobacterium tuberculosis

25

<400> 112
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<211> 115

45 <212> PRT

<213> Mycobacterium tuberculosis

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<400> 113
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1 5 10 15

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5	Arg Glu His Leu Ile Glu Ser Pro Thr Thr Ser Ile Cys Pro Trp Lys			
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	Gly Leu Ala His Tyr Tyr Asn Val Val Val Asp Gly Pro Tyr Gly Pro			
	50	55	60	
10	Val Asn Pro Asp Ala Ala Trp Tyr Tyr Arg Arg Pro Ser Pro Leu Ala			
	65	70	75	80
15	Arg Arg Ile Lys Asn His Val Ala Phe Trp His Gly Val Thr Val Glu			
		85	90	95
	Gly Glu Ser Glu Ser Arg His Gly Leu Ala Arg Arg Val Val Ala Trp			
		100	105	110
20	Leu Gly Lys			
		115		
25	<210> 114			
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	<212> DNA			
30	<213> Mycobacterium tuberculosis			
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	accacgtcga tatgcccatg gaagggctctg gccattact acaacgtcgt cgtggacggc	180		
	ccctatggtc cggttaaccc ggacgctgcc tggtaactacc gccggcccag tccactggct	240		
40	cgccggatca aaaaccatgt tgcgttcttg cacggtgtga cggtcgaagg tgaatccgag	300		
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50	<213> Mycobacterium tuberculosis			
	<400> 115			
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	1 5 10 15			

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 20 25 30
 5 Pro Leu Arg Phe Thr Glu Ile Glu Arg Gly Leu Pro Gly Ile Ser Arg
 35 40 45
 Ser Val Leu Ala Gln Arg Leu Arg Arg Leu Gln His Asp Arg Ile Ile
 50 55 60
 10 Glu Ala Val Pro Glu His Thr Gly Gly Gly Tyr Arg Phe Thr Val Ala
 65 70 75 80
 15 Gly Glu Glu Leu Arg Pro Val Leu Gln Thr Leu Gly Asp Trp Val Ser
 85 90 95
 Arg Trp Leu Met Ala Asp Pro Thr Pro Ala Glu Cys Asp Pro Glu Leu
 100 105 110
 20 Leu Thr Leu Trp Ile Ser Arg Arg Val Asn Thr Glu Ala Leu Pro Gly
 115 120 125
 25 Arg Arg Val Val Val Glu Phe Arg Tyr His Gly Glu Arg Pro Leu Trp
 130 135 140
 Ala Trp Leu Val Leu Glu Pro Gly Asp Ile Ser Val Cys Leu His Asp
 145 150 155 160
 30 Pro Cys Leu Pro Val Asp Leu Thr Val Arg Gly His Pro Arg Asp Leu
 165 170 175
 35 Tyr Arg Val Tyr Ser Gly Arg Ser Thr Leu Ala Ala Glu Ile Ser Ala
 180 185 190
 Glu Arg Ile Glu Leu Asp Gly Leu Pro Ala Met Arg Arg Ala Phe Pro
 195 200 205
 40 Ser Trp Met Ala Trp Ser Pro Phe Ala Pro Ala Met Arg Gln Ala Val
 210 215 220
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 225 230 235
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 <211> 708
 50 <212> DNA
 <213> Mycobacterium tuberculosis
 55 <400> 116

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<210> 117

<211> 517

<212> PRT

<213> Mycobacterium tuberculosis

<400> 117

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Leu Asp Arg Leu Pro His Glu Ile Ala Thr Ala Val Val Glu Thr Val
 35 40 45

Asn Cys Ala Asp Pro Gly Ala Ala Phe Asp Glu Leu Asp Ala Lys Ile
 50 55 60

Asn Ala Gly Met Lys Ala Tyr Ala Ile Pro Gly Val Ala Val Ala Val
 65 70 75 80

Trp Ala Gly Gly Gln Glu Tyr Val Lys Gly Tyr Gly Val Thr Asn Val
 85 90 95

Asp His Pro Met Pro Val Asp Gly Asp Thr Val Phe Arg Ile Gly Ser
 100 105 110

Thr Thr Lys Thr Phe Thr Gly Thr Val Met Met Arg Leu Val Glu Arg

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	115		120		125												
5	Gly	Lys	Val	Asp	Leu	Asp	Ser	Pro	Val	Arg	Arg	Tyr	Ile	Pro	Asp	Phe	
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10	Leu	Asn	His	Thr	Ala	Gly	Trp	Asp	Gly	Arg	Asn	Gly	Gln	Asp	Phe	Gly	
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35			275					280					285				
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40	Asn	Gly	Glu	Gln	Ile	Leu	Ser	Arg	Gln	Ser	Leu	Lys	Ala	Met	Arg	Ser	
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				340					345					350			
50	His	Gly	Gly	Thr	Trp	Lys	Gly	Gln	Arg	Ser	Gly	Phe	Val	Met	Val	Pro	
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	Asp	Arg	Asn	Phe	Ala	Met	Thr	Val	Leu	Thr	Asn	Ser	Asp	Gly	Gly	Phe	
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	His Met Ile Asn Asp Leu Phe Ala Ser Asp Trp Ala Leu Gln Arg Phe	
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	Asp Leu Ala Pro Tyr Glu Gly Arg Tyr Ile Ala Lys Gln Val Ala Gln	
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10	Asn Gly Asp Leu Glu Thr Thr Val Ile Asp Phe Arg Ala Arg Asp Gly	
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	Gln Leu Ala Gly Ser Met Ser Thr Asp Asp Ala Asn Pro Asp Gly Gln	
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55	gcggtggcgc tctatgtcaa ggcgatgaca cgcctaccgc agctcacccc tccgggaacc	600

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<211> 108

<212> PRT

<213> Mycobacterium tuberculosis

<400> 119

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35 40 45

Thr Val Arg Lys Trp Val Arg Gln Ala Gln Val Asp Ala Gly Ala Arg
50 55 60

Pro Gly Thr Thr Thr Glu Glu Ser Ala Glu Leu Lys Arg Leu Arg Arg
65 70 75 80

Asp Asn Ala Glu Leu Arg Arg Ala Asn Ala Ile Leu Lys Thr Ala Ser
85 90 95

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<210> 120

<211> 324

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<212> DNA

<213> Mycobacterium tuberculosis

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<211> 365

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<212> PRT

<213> Mycobacterium tuberculosis

35

<400> 121

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20 25 30

Asn Ala Ile Asp Gly Arg Ile Val Glu Ile Val Ala Glu Leu Asp Arg
35 40 45

45

Asp Gly Leu Trp Gly Val Thr Gly Ala Arg Ser Val Ala Gly Leu Val
50 55 60

Ala Trp Lys Met Gly Cys Ser Ser Gly Asn Ala His Thr Ile Ala Thr
65 70 75 80

Val Ala Arg Arg Leu Pro Glu Phe Pro Arg Cys Ala Arg Gly Met Arg
85 90 95

55

Glu Gly Arg Leu Ser Leu Asp Gln Val Gly Val Ile Ala Gly Arg Ala

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	Val Asn Gln Leu Arg Thr Ala Leu Lys Leu Glu Pro Arg Pro Glu Pro 130 135 140		
10	Glu Pro Asp Phe Arg Pro Glu Pro Arg Pro Ser Ile Thr Arg Ser Ala 145 150 155 160		
15	Asp Glu Gln Phe Ser Cys Trp Arg Ile Lys Leu Pro His Val Glu Ala 165 170 175		
	Ala Lys Phe Asp Ala Ala Leu Gln Ser His Leu Asp Ala Leu Ile Ala 180 185 190		
20	Glu Tyr Lys Arg Asp His Asp Asn Ser Asp Gly Val Ser Asp Gln Arg 195 200 205		
25	Pro Pro Leu Pro Gly Asn Val Glu Ala Phe Leu Arg Leu Val Glu Ala 210 215 220		
	Gly Trp Asp Ala Glu Val Ala Arg Arg Pro His Gly Gln His Thr Thr 225 230 235 240		
30	Val Val Met His Leu Asp Val Gln Glu Arg Ala Ala Gly Leu His Leu 245 250 255		
35	Gly Pro Leu Leu Ser Glu Ser Glu Arg Arg Tyr Leu Leu Cys Asp Ala 260 265 270		
	Thr Phe Glu Ala Trp Phe Glu Arg Asp Gly Gln Val Ile Gly Cys Gly 275 280 285		
40	Arg Thr Thr Arg Gln Ile Asn Arg Arg Leu Arg Arg Ala Leu Glu His 290 295 300		
45	Arg Asp Arg Thr Cys Val Val Pro Gly Cys Gly Ala Thr Arg Gly Leu 305 310 315 320		
	His Ala His His Ile Arg His Trp Gln Asp Gly Gly Ala Thr Glu Leu 325 330 335		
50	Ala Asn Leu Val Leu Val Cys Pro Tyr His His Arg Ala His His Arg 340 345 350		
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 <211> 1095
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45 <210> 123
 <211> 434
 <212> PRT
 <213> Mycobacterium tuberculosis

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 1 5 10 15

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			35					40					45			
10	Pro	Tyr	Pro	Leu	Tyr	Glu	Arg	Met	Arg	Ala	Ala	Gly	Ser	Val	His	Arg
		50					55					60				
	Ile	Ala	Asn	Ser	Asp	Phe	Tyr	Ala	Val	Cys	Gly	Trp	Asp	Ala	Val	Asn
	65					70					75					80
15	Glu	Ala	Ile	Gly	Arg	Pro	Glu	Asp	Phe	Ser	Ser	Asn	Leu	Thr	Ala	Thr
					85					90					95	
	Met	Thr	Tyr	Thr	Ala	Glu	Gly	Thr	Ala	Lys	Pro	Phe	Glu	Met	Asp	Pro
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	Leu	Gly	Gly	Pro	Thr	His	Val	Leu	Ala	Thr	Ala	Asp	Asp	Pro	Ala	His
			115					120					125			
25	Ala	Val	His	Arg	Lys	Leu	Val	Leu	Arg	His	Leu	Ala	Ala	Lys	Arg	Ile
		130					135					140				
	Arg	Val	Met	Glu	Gln	Phe	Thr	Val	Gln	Ala	Ala	Asp	Arg	Leu	Trp	Val
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	Asp	Gly	Met	Gln	Asp	Gly	Cys	Ile	Glu	Trp	Met	Gly	Ala	Met	Ala	Asn
				165						170					175	
35	Arg	Leu	Pro	Met	Met	Val	Val	Ala	Glu	Leu	Ile	Gly	Leu	Pro	Asp	Pro
			180						185					190		
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40			195					200					205			
	Glu	Gly	Leu	Val	Glu	Asn	Asp	Gln	Leu	Val	Ala	Ala	Gly	Val	Ala	Leu
		210					215					220				
45	Met	Glu	Leu	Ser	Gly	Tyr	Ile	Phe	Glu	Gln	Phe	Asp	Arg	Ala	Ala	Ala
	225					230					235					240
	Asp	Pro	Arg	Asp	Asn	Leu	Leu	Gly	Glu	Leu	Ala	Thr	Ala	Cys	Ala	Ser
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 Glu Leu Leu Gly Ala Phe Ile Glu Glu Thr Leu Arg Tyr Glu Pro Pro
 305 310 315 320
 10 Phe Arg Gly His Tyr Arg His Val Arg Asn Ala Thr Thr Leu Asp Gly
 325 330 335
 Thr Glu Leu Pro Ala Asp Ser His Leu Leu Leu Leu Trp Gly Ala Ala
 340 345 350
 15 Asn Arg Asp Pro Ala Gln Phe Glu Ala Pro Gly Glu Phe Arg Leu Asp
 355 360 365
 20 Arg Ala Gly Gly Lys Gly His Ile Ser Phe Gly Lys Gly Ala His Phe
 370 375 380
 Cys Val Gly Ala Ala Leu Ala Arg Leu Glu Ala Arg Ile Val Leu Arg
 385 390 395 400
 25 Leu Leu Leu Asp Arg Thr Ser Val Ile Glu Ala Ala Asp Val Gly Gly
 405 410 415
 30 Trp Leu Pro Ser Ile Leu Val Arg Arg Ile Glu Arg Leu Glu Leu Ala
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 40 <213> Mycobacterium tuberculosis
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Ala Leu Thr Ala Thr Gln Phe Asn Phe His Ala Ala Met Tyr Gln Ala
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Val Gly Ala Gln Ala Ile Ala Met Asn Glu Ala Phe Val Ala Met Leu
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Ala Val Ser

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45 Val Leu Asn Pro Leu Asp Glu Ser Asp Arg Arg Arg Phe Leu Asp Leu
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Ala Ala Ser Ala Asp Ile Val Val Asp Cys Gly Leu Pro Gly Gln Ala
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 Phe Lys Pro Gly Thr Leu Thr Ser Leu Gly Phe Ser Tyr Asp Val Leu
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<212> DNA

<213> Mycobacterium tuberculosis

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<213> Mycobacterium tuberculosis

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<400> 131

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Gln Asn Cys Ser Thr Thr Trp Pro Lys Leu Arg Asp Arg Pro Arg Leu
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Gly Val Ser Val Leu Ala Glu Gly His Asp Ala Ala Cys Met Ser Leu
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Ser Arg Lys Glu Gly Asn Arg Phe Ala Gly Val Phe Trp Ser Glu Leu
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Ser Ser Gly Gly Val Val Ile Ala Gly Ala Gly Ala Trp Leu Asp Cys
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<211> 513

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<213> Mycobacterium tuberculosis

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<212> PRT

<213> Mycobacterium tuberculosis

<400> 135

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Pro Glu Gly Arg Gly Val Leu Gly Ala Leu Ile Glu Glu Pro Lys Pro
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Ile Arg Leu Asp Asp Ile Ser Arg His Pro Ala Ser Val Gly Phe Pro
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Arg Asp Glu Val Phe Gly Asn Leu Tyr Leu Thr Glu Lys Ala Asp Gly
165 170 175

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Gln Pro Phe Ser Asp Asp Asp Glu Val Leu Val Gln Ala Leu Ala Ala
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Ala Ala Gly Ile Ala Val Asp Asn Ala Arg Leu Phe Glu Glu Ser Arg
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Thr Arg Glu Ala Trp Ile Glu Ala Thr Arg Asp Ile Gly Thr Gln Met
210 215 220

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Leu Ala Gly Ala Asp Pro Ala Met Val Phe Arg Leu Ile Ala Glu Glu

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<212> DNA

<213> Mycobacterium tuberculosis

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 <212> PRT
 25 <213> Mycobacterium tuberculosis
 30
 <400> 137
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 35 Val Arg Pro Ala Thr Pro Leu Ile Cys Ala Phe Gly Asp Lys His Lys
 20 25 30
 His Thr Tyr Gly Val Thr Pro Ile Cys Arg Ala Leu Ala Val His Gly
 35 40 45
 40 Val Gln Ile Ala Ser Arg Thr Tyr Phe Ala Asp Arg Ala Ala Ala Pro
 50 55 60
 45 Ser Lys Arg Ala Leu Trp Asp Thr Thr Ile Thr Glu Ile Leu Ala Gly
 65 70 75 80
 Tyr Tyr Glu Pro Asp Ala Glu Gly Lys Arg Pro Pro Glu Cys Leu Tyr
 85 90 95
 50 Gly Ser Leu Lys Met Trp Ala His Leu Gln Arg Gln Gly Phe Arg Trp
 100 105 110
 55 Pro Ser Ala Thr Val Lys Thr Ile Met Arg Ala Asn Gly Trp Arg Gly
 115 120 125

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Val Pro Leu Ala Ala His Ile Thr His His Arg Thr Arg Pro Gly Arg
130 135 140

5

Gly Pro Gly Pro Arg Pro Gly Gly Ser Ala Met Ala Gly Phe Ser Asn
145 150 155 160

10

Glu Pro Ala Gly Ser Gly Arg Leu His Leu Arg Ala Asp Asp Val Glu
165 170 175

Phe Arg Leu His Arg Val Arg Gly Arg Arg Leu Arg Arg Cys Asp Arg
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Gly Leu Gly Met Leu Ala Asp Gln Arg Arg Ser Val Arg Arg Thr Arg
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<210> 138

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<212> DNA

<213> Mycobacterium tuberculosis

30

<400> 138

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agacccggcc gcggcccagg ccctagacct ggcggttcgg caatggcggg ctttagcaac 480

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cgcgttcgtg gtcgacgcct acgcgggtgt gatcgcgggc tgggaatgct cgttgaccaa 600

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<211> 121

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20 25 30

Gly Ser Gln Tyr Thr Ala Ile Tyr Phe Gly Lys Thr Pro Met Leu Ala
35 40 45

15 Gly Leu Arg Pro Ser Ile Gly Ile Val Gly Asp Ala Leu Asp Asn Ala
50 55 60

20 Leu Cys Glu Thr Thr Thr Gly Pro His Arg Thr Glu Cys Ser His Gly
65 70 75 80

Ser Pro Phe Arg Ser Gly Pro Ile Arg Thr Leu Ala Asp Leu Glu Asp
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30 Arg Ile Pro Gly Arg Leu Gln Pro Ala
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<210> 140

<211> 363

35 <212> DNA

<213> Mycobacterium tuberculosis

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45 ttccggcaaga caccgatgct agccgggctg cggccgtcga taggcattgt tggcgacgcc 180

ctcgacaacg ccttatgtga aaccacgaca gggccccaca ggaccgaatg cagccacggc 240

agcccggttc gtagcggggc gatccgcacc ctggtgacc tggaagacat cgccctcggcg 300

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<210> 141

<211> 375

55 <212> PRT

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<213> Mycobacterium tuberculosis

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<400> 141

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20 25 30

15

Thr Pro Gly Tyr Asp Leu Cys Tyr Leu Ile Gly Ser Arg Ala Glu Thr
35 40 45

Phe Glu Arg Leu Thr Ala Leu Val Leu Pro Ala Ala Gly Ala Pro Ala
50 55 60

20

Val Val Leu Pro Arg Leu Glu Leu Ala Ala Leu Lys Gln Ser Ala Ala
65 70 75 80

25

Ala Glu Leu Gly Leu Arg Val Cys Asp Trp Val Asp Gly Asp Asp Pro
85 90 95

Tyr Gly Leu Val Ser Ala Val Leu Gly Gly Ala Pro Val Ala Thr Ala
100 105 110

30

Val Thr Asp Ser Met Pro Ala Leu His Met Leu Pro Leu Ala Asp Ala
115 120 125

35

Leu Gly Val Leu Pro Val Leu Ala Thr Asp Val Leu Arg Arg Leu Arg
130 135 140

Met Val Lys Glu Glu Thr Glu Ile Asp Ala Leu Arg Lys Ala Gly Ala
145 150 155 160

40

Ala Ile Asp Arg Val His Ala Arg Val Pro Glu Phe Leu Val Pro Gly
165 170 175

45

Arg Thr Glu Ala Asp Val Ala Ala Asp Ile Ala Glu Ala Ile Val Ala
180 185 190

Glu Gly His Ser Glu Val Ala Phe Val Ile Val Gly Ser Gly Pro His
195 200 205

50

Gly Ala Asp Pro His His Gly Tyr Ser Asp Arg Glu Leu Arg Glu Gly
210 215 220

55

Asp Ile Val Val Val Asp Ile Gly Gly Thr Tyr Gly Pro Gly Tyr His
225 230 235 240

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20	Gly	Asn	Asp	Leu	Val	Leu	Val	Pro	Gly	Met	Ala	Phe	Ser	Ile	Glu	Pro	
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40	<400> 142																
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 20 <211> 244
 <212> PRT
 <213> Mycobacterium tuberculosis

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 35 Pro Trp Gln Gln Gln Pro Thr Gln Glu Ala Thr Trp Gln Ala Pro Ala
 35 40 45
 40 Tyr Thr Pro Gln Tyr Gln Gln Pro Ala Asp Pro Ala Tyr Pro Gln Gln
 50 55 60
 Tyr Pro Gln Pro Thr Pro Gly Tyr Ala Gln Pro Glu Gln Phe Gly Ala
 65 70 75 80
 45 Gln Pro Thr Gln Leu Gly Val Pro Gly Gln Tyr Gly Gln Tyr Gln Gln
 85 90 95
 50 Pro Gly Gln Tyr Gly Gln Pro Gly Gln Tyr Gly Gln Pro Gly Gln Tyr
 100 105 110
 Ala Pro Pro Gly Gln Tyr Pro Gly Gln Tyr Gly Pro Tyr Gly Gln Ser
 115 120 125
 55 Gly Gln Gly Ser Lys Arg Ser Val Ala Val Ile Gly Gly Val Ile Ala
 130 135 140

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5	Val Met Ala Val Leu Phe Ile Gly Ala Val Leu Ile Leu Gly Phe Trp 145 150 155 160	
	Ala Pro Gly Phe Phe Val Thr Thr Lys Leu Asp Val Ile Lys Ala Gln 165 170 175	
10	Ala Gly Val Gln Gln Val Leu Thr Asp Glu Thr Thr Gly Tyr Gly Ala 180 185 190	
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	Lys Lys Gly Ala Thr Phe Glu Cys Thr Val Ser Ile Asp Gly Thr Ser 210 215 220	
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	aacaacgggt cagaccccac ggtcaaaaag ggcgccacct tcgaatgcac ggtgagcatc	660
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 <213> Mycobacterium tuberculosis
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 <400> 145
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 20 25 30
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 35 40 45
 20 Thr Ile Phe Gly Phe Val Trp Tyr Ser His Ser Ile Phe Gly Leu Asp
 50 55 60
 25 Ser Leu Gly Glu Trp Leu Arg His Pro Tyr Cys Ala Leu Pro Gln Ser
 65 70 75 80
 30 Ala Arg Ala Asp Ile Ser Ala Asp Gly Glu Cys Arg Leu Leu Ala Thr
 85 90 95
 Ala Pro Phe Asp Gln Phe Met Leu Arg Leu Lys Val Gly Met Ala Ala
 100 105 110
 35 Gly Ile Val Leu Ala Cys Pro Val Trp Phe Tyr Gln Leu Trp Ala Phe
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 40 Ile Thr Pro Gly Leu Tyr Gln Arg Glu Arg Arg Phe Ala Val Ala Phe
 130 135 140
 Val Ile Pro Ala Ala Val Leu Phe Val Ala Gly Ala Val Leu Ala Tyr
 145 150 155 160
 45 Leu Val Leu Ser Lys Ala Leu Gly Phe Leu Leu Thr Val Gly Ser Asp
 165 170 175
 50 Val Gln Val Thr Ala Leu Ser Gly Asp Arg Tyr Phe Gly Phe Leu Leu
 180 185 190
 Asn Leu Leu Val Val Phe Gly Val Ser Phe Glu Phe Pro Leu Leu Ile
 195 200 205
 55 Val Met Leu Asn Leu Ala Gly Leu Leu Thr Tyr Glu Arg Leu Lys Ser
 210 215 220

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5 Trp Arg Arg Gly Leu Ile Phe Ala Met Phe Val Phe Ala Ala Ile Phe
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 Thr Pro Gly Ser Asp Pro Phe Ser Met Thr Ala Leu Gly Ala Ala Leu
 245 250 255
 10 Thr Val Leu Leu Glu Leu Ala Ile Gln Ile Ala Arg Val His Asp Lys
 260 265 270
 Arg Lys Ala Lys Arg Glu Ala Ala Ile Pro Asp Asp Glu Ala Ser Val
 275 280 285
 15 Ile Asp Pro Pro Ser Pro Val Pro Ala Pro Ser Val Ile Gly Ser His
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 20 Asp Asp Val Thr
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 <210> 146
 <211> 924
 25 <212> DNA
 <213> Mycobacterium tuberculosis
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 <212> PRT
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<400> 147

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 20 25 30

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 35 40 45

Thr Val Arg Lys Trp Val Arg Gln Ala Gln Val Asp Ala Gly Ala Arg
 50 55 60

25

Pro Gly Thr Thr Thr Glu Glu Ser Ala Glu Leu Lys Arg Leu Arg Arg
 65 70 75 80

30

Asp Asn Ala Glu Leu Arg Arg Ala Asn Ala Ile Leu Lys Thr Ala Ser
 85 90 95

Ala Phe Phe Ala Ala Glu Leu Asp Arg Pro Ala Arg
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<210> 148

<211> 324

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<212> DNA

<213> Mycobacterium tuberculosis

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50

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gccggcgcac ggcccgggac caccgacgaa gaatccgctg agctgaagcg cttgcggcgg 240

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 35 40 45
 20 Thr Val Arg Lys Trp Val Arg Gln Ala Gln Val Asp Ala Gly Ala Arg
 50 55 60
 Pro Gly Thr Thr Thr Glu Glu Ser Ala Glu Leu Lys Arg Leu Arg Arg
 25 65 70 75 80
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 100 105

 35 <210> 150
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 gccgagctcg accggccagc acgc 324

 55 <210> 151
 <211> 414

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<212> PRT

<213> Mycobacterium tuberculosis

<400> 151

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Glu Leu Leu Asp Thr Val Pro Asp Ser Leu Leu Arg Arg Leu Lys Gln
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15

Tyr Ser Gly Arg Leu Ala Thr Glu Ala Val Ser Ala Met Gln Glu Arg
35 40 45

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Leu Pro Phe Phe Ala Asp Leu Glu Ala Ser Gln Arg Ala Ser Val Ala
50 55 60

Leu Val Val Gln Thr Ala Val Val Asn Phe Val Glu Trp Met His Asp
65 70 75 80

25

Pro His Ser Asp Val Gly Tyr Thr Ala Gln Ala Phe Glu Leu Val Pro
85 90 95

30

Gln Asp Leu Thr Arg Arg Ile Ala Leu Arg Gln Thr Val Asp Met Val
100 105 110

Arg Val Thr Met Glu Phe Phe Glu Glu Val Val Pro Leu Leu Ala Arg
115 120 125

35

Ser Glu Glu Gln Leu Thr Ala Leu Thr Val Gly Ile Leu Lys Tyr Ser
130 135 140

40

Arg Asp Leu Ala Phe Thr Ala Ala Thr Ala Tyr Ala Asp Ala Ala Glu
145 150 155 160

Ala Arg Gly Thr Trp Asp Ser Arg Met Glu Ala Ser Val Val Asp Ala
165 170 175

45

Val Val Arg Gly Asp Thr Gly Pro Glu Leu Leu Ser Arg Ala Ala Ala
180 185 190

50

Leu Asn Trp Asp Thr Thr Ala Pro Ala Thr Val Leu Val Gly Thr Pro
195 200 205

Ala Pro Gly Pro Asn Gly Ser Asn Ser Asp Gly Asp Ser Glu Arg Ala
210 215 220

55

Ser Gln Asp Val Arg Asp Thr Ala Ala Arg His Gly Arg Ala Ala Leu

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10	Ala Pro Val Val Ile Gly Pro Thr Ala Pro Met Leu Thr Ala Ala His	275		280		285	
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	Arg Gly Ala Pro Arg Pro Val Leu Ala Arg Glu Leu Leu Pro Glu Arg	305		310		315	320
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25	Met Arg Pro Leu Ala Asp Ala Gly Pro Thr Leu Ile Glu Thr Leu Asp	340		345		350	
	Ala Tyr Leu Asp Cys Gly Gly Ala Ile Glu Ala Cys Ala Arg Lys Leu	355		360		365	
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	ccgcacagtg acgtcggeta taccgcgcag gcattcgagc tgggtgcccc ggatctgacg					300	

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 25 attgaagctt gtgccagaaa gttgttcgtt catccaaaca cagtgcggta ccggctcaag 1140
 cggatcacgg acttcaccgg gcgcgatccc accagccac gcgatgccta tgctcttcgg 1200
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30 <210> 153

<211> 312

<212> PRT

35 <213> Mycobacterium tuberculosis

<400> 153

40 Met Pro Leu Ser Ser Arg Met Pro Gly Leu Thr Cys Phe Glu Ile Phe
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Leu Ala Ile Ala Glu Ala Gly Ser Leu Gly Gly Ala Ala Arg Glu Leu
20 25 30

45 Gly Leu Thr Gln Gln Ala Val Ser Arg Arg Leu Ala Ser Met Glu Ala
35 40 45

50 Gln Ile Gly Val Arg Leu Ala Ile Arg Thr Thr Arg Gly Ser Gln Leu
50 55 60

Thr Pro Ala Gly Ile Val Val Ala Glu Trp Ala Ala Arg Leu Leu Glu
65 70 75 80

55 Val Ala Asp Glu Ile Asp Ala Gly Leu Gly Ser Leu Arg Thr Glu Gly

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	85								90				95			
5	Arg	Gln	Arg	Ile 100	Arg	Val	Val	Ala	Ser 105	Gln	Thr	Ile	Ala	Glu 110	Gln	Leu
	Met	Pro	His 115	Trp	Met	Leu	Ser	Leu 120	Arg	Ala	Ala	Asp	Met 125	Arg	Arg	Gly
10	Gly	Thr 130	Val	Pro	Glu	Val	Ile 135	Leu	Thr	Ala	Thr	Asn 140	Ser	Glu	His	Ala
15	Ile 145	Ala	Ala	Val	Arg	Asp 150	Gly	Ile	Ala	Asp	Leu 155	Gly	Phe	Ile	Glu	Asn 160
	Pro	Cys	Pro	Pro	Thr 165	Gly	Leu	Gly	Ser	Val 170	Val	Val	Ala	Arg	Asp 175	Glu
20	Leu	Val	Val	Val 180	Val	Pro	Pro	Gly	His 185	Lys	Trp	Ala	Arg	Arg 190	Ser	Arg
	Val	Val	Ser 195	Ala	Arg	Glu	Leu	Ala 200	Gln	Thr	Pro	Leu	Val 205	Thr	Arg	Glu
25	Pro	Asn 210	Ser	Gly	Ile	Arg	Asp 215	Ser	Leu	Thr	Ala	Ala 220	Leu	Arg	Asp	Thr
30	Leu 225	Gly	Glu	Asp	Met	Gln 230	Gln	Ala	Pro	Pro	Val 235	Leu	Glu	Leu	Ser	Ser 240
	Ala	Ala	Ala	Val	Arg 245	Ala	Ala	Val	Leu	Ala 250	Gly	Ala	Gly	Pro	Ala 255	Ala
35	Met	Ser	Arg	Leu 260	Ala	Ile	Ala	Asp	Asp 265	Leu	Ala	Phe	Gly	Arg 270	Leu	Leu
40	Ala	Val	Asp 275	Ile	Pro	Ala	Leu	Asn 280	Leu	Arg	Arg	Gln	Leu 285	Arg	Ala	Ile
	Trp	Val 290	Gly	Gly	Arg	Thr	Pro 295	Pro	Ala	Gly	Ala	Ile 300	Arg	Asp	Leu	Leu
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50	<210>		154													
	<211>		936													
	<212>		DNA													
55	<213>		Mycobacterium tuberculosis													

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 aggcggctcg catcgatgga ggcccagatc ggggtgcgat tggccatccg gacgacacgt 180
 10 ggctcccaac tcacgcctgc cggcatcgtc gtcgccgaat gggcgggccg cttgctcgaa 240
 gtcgccgacg agatcgatgc cggcctcggc tcgctgcgca ccgaaggccg ccagcgcacg 300
 agagtggtag ccagccagac gatagccgaa cagctgatgc cgcattggat gctgtccttg 360
 cgggcggccg acatgcgccg cgggtggtag gtccctgagg taatcctgac cgccaccaat 420
 15 agcgagcatg cgatcgagc cgttcgtgac ggcatcgag atcttggatt catcgaaaac 480
 cctgtcctc ccacgggatt aggcagcgtt gtggttgca cgcaggaact ggtcgtcgtc 540
 gtgcccggcg gtcacaagtg gggccgacgg tcacgagtag tgagcgcccg ggagctcgct 600
 20 cagacgcctt tggtagctcg cgaaccgaac tctggcatcc gcgattcact caccgcggcg 660
 ttacgtgaca cgctcgggga ggacatgcag caagcgccac cggtagctgga attatcatcg 720
 gctcggcccg tgcggggccg ggtcttgcc ggcgtggac cggctgcgat gagccggcta 780
 25 gcgtagccg atgacctggc gttcggtcga ttactcggcg tcgacatccc cgcgttgaac 840
 ctgcggcgcc agcttcgagc catctgggtc ggtggcgca ccccgccgcg gggtagcata 900
 cgagacctgc tcagccacat cacttccgcg agcacg 936
 30
 <210> 155
 <211> 74
 <212> PRT
 35 <213> Mycobacterium tuberculosis
 40
 <400> 155
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 1 5 10 15
 45 Ala Val Ala Asp Ala His Gly Val Pro Phe Leu Gly Ile Arg Gly Met
 20 25 30
 Ser Asp Gly Pro Gly Asp Pro Leu His Leu Pro Gly Phe Pro Val Gln
 35 40 45
 50 Phe Phe Val Tyr Lys Gln Ile Ala Ala Asn Asn Ala Ala Arg Val Thr
 50 55 60
 55 Glu Ala Phe Leu Gln Asn Trp Ala Gly Val
 65 70

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5 <210> 156
 <211> 222
 <212> DNA
 <213> Mycobacterium tuberculosis

10 <400> 156
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 gcacacggcg tcccgttcct gggaattcgc ggtatgtccg acgggcccgg cgaccgctg 120
 catctgccgg gcttccccgt ccagttcttc gttacaagc agattgcggc caacaacgcc 180
 15 gcccgggtea ccgaagcctt cctgcagaac tgggcccggc tc 222

20 <210> 157
 <211> 114
 <212> PRT
 <213> Mycobacterium tuberculosis

25 <400> 157
 Val Val Ala Ala Leu His Ala Gly Lys Ala Val Thr Ile Ala Pro Gln
 1 5 10 15
 Ser Met Thr Leu Thr Thr Gln Gln Ala Ala Asp Leu Leu Gly Val Ser
 20 25 30
 35 Arg Pro Thr Val Val Arg Leu Ile Lys Ser Gly Glu Leu Ala Ala Glu
 35 40 45
 Arg Ile Gly Asn Arg His Arg Leu Val Leu Asp Asp Val Leu Ala Tyr
 50 55 60
 40 Arg Glu Ala Arg Arg Gln Arg Gln Tyr Asp Ala Leu Ala Glu Ser Ala
 65 70 75 80
 45 Met Asp Ile Asp Ala Asp Glu Asp Pro Glu Val Ile Cys Glu Gln Leu
 85 90 95
 Arg Glu Ala Arg Arg Val Val Ala Ala Arg Arg Arg Thr Glu Arg Arg
 100 105 110
 50 Arg Ala

55 <210> 158

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<211> 342

<212> DNA

5 <213> Mycobacterium tuberculosis

<400> 158

10 gtggtggctg cgctgcacgc cggcaaggcg gtgaccatcg cgccgcagag catgacgctg 60
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aagagcggcg agctggccgc cgagcgcacg gggaatcgcc accggctcgt gctcgacgac 180
15 gtgttggcct accgggaggc ccgccggcag cgccagtacg acgcgcttgc cgagagcgca 240
atggacatcg acgccgacga ggatcccgag gtgatttgcg agcagttgcg tgaggcgcg 300
cgtgttgctg ccgcgcgccg tagaactgag cggcggcgcg cc 342

20 <210> 159

<211> 221

<212> PRT

25 <213> Mycobacterium tuberculosis

<400> 159

30 Met Thr Asn Leu Ala Asp Ala Thr Gln Ala Thr Met Ala Leu Val Glu
1 5 10 15

Arg His Ala Ala His Asn Tyr Ser Pro Leu Pro Val Val Ala Ala Ser
20 25 30

Ala Glu Gly Ala Trp Ile Ala Asp Ile Asp Gly Leu Arg Tyr Leu Asp
35 40 45

40 Trp Leu Ala Ala Tyr Ser Ala Val Asn Leu Gly His Arg Asn Pro Ala
50 55 60

Ser Thr Ala Thr Ala His Ala Gln Val Asp Thr Val Thr Leu Leu Asn
45 65 70 75 80

Arg Ala Leu His Ala Asp Arg Leu Gly Pro Leu Gly Ala Ala Leu Ala
85 90 95

50 Gln Leu Cys Gly Lys Asp Val Val Leu Pro Met Asn Ser Asp Ala Glu
100 105 110

Ala Val Glu Ser Gly Leu Arg Val Ala Arg Lys Trp Gly Ala Asp Val
115 120 125

55 Asn Gly Leu Pro Ala Gly Arg His Asp Ile Ile Leu Ala Asn Asn Asn

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	130	135	140	
5	Phe His Gly His Thr Ser Ser Val Val Ser Phe Ser Ser Asp Pro Ala 145 150 155 160			
	Ala Gly Ser Gly Val Glu Pro Ser Thr Pro Gly Leu Arg Ser Val Pro 165 170 175			
10	Phe Gly Asp Ala Ala Ala Pro Ala Gln Thr Ile Asp Asp Asn Thr Val 180 185 190			
15	Ala Asp Leu Leu Glu Pro Ile Pro Gly Gln Ala Gly Ile Ile Val Pro 195 200 205			
	Ala Asp Asp Tyr Leu Pro Ala Ala Ser Ser Thr Thr Cys 210 215 220			
20	<210> 160			
	<211> 663			
25	<212> DNA			
	<213> Mycobacterium tuberculosis			
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	cacaattatt cgccgctgcc tgtggtggcg gccagcgctg aggggtgcgtg gatcgccgat 120			
	atcgacggcc tgcgctacct ggactggctg gctgcgtact cggcggtaaa ccttggccat 180			
35	cgcaaccccc cgagcaccgc cacggctcat gcccaagtcg acaccgtcac cctgctgaat 240			
	cgggccttgc atgccgaccg actcggggcg ttgggcgcgc cgcttgccca gctgtgcggc 300			
	aaagacgtgg tgttgccaat gaactctgat gctgaagcgg tggagagcgg tcttagggtc 360			
40	gcccgcgaagt ggggagccga cgtaacggc ctccccgcgg gccggcacga tatcattttg 420			
	gcaaacaaca actttcatgg ccacaccagc agtgctcgta gcttctcgtc ggaccgcgct 480			
	gcgggcagcg gcgtcgaacc ttctaccccg ggactccgct cggtaccgtt tggcgatgct 540			
45	gcggcaccgg cgagacaat cgacgacaac accgtcgctg acctgctcga gccgattccc 600			
	ggccaggcgg gcatcatcgt cccggccgac gactacctgc cggctgcgtc gagcacaacg 660			
	tgc 663			
50	<210> 161			
	<211> 302			
55	<212> PRT			
	<213> Mycobacterium tuberculosis			

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5 <400> 161
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 1 5 10 15
 10 Tyr Arg Trp Phe Met Thr Asp Ser Tyr Val Ala Ala Ala Arg Leu Gly
 20 25 30
 Ser Pro Ala Arg Arg Thr Pro Arg Thr Arg Arg Tyr Ala Met Thr Pro
 35 40 45
 15 Pro Ala Phe Phe Ala Val Ala Tyr Ala Ile Asn Pro Trp Met Asp Val
 50 55 60
 20 Thr Ala Pro Val Asp Val Gln Val Ala Gln Ala Gln Trp Glu His Leu
 65 70 75 80
 His Gln Thr Tyr Leu Arg Leu Gly His Ser Val Asp Leu Ile Glu Pro
 85 90 95
 25 Ile Ser Gly Leu Pro Asp Met Val Tyr Thr Ala Asn Gly Gly Phe Ile
 100 105 110
 30 Ala His Asp Ile Ala Val Val Ala Arg Phe Arg Phe Pro Glu Arg Ala
 115 120 125
 Gly Glu Ser Arg Ala Tyr Ala Ser Trp Met Ser Ser Val Gly Tyr Arg
 130 135 140
 35 Pro Val Thr Thr Arg His Val Asn Glu Gly Gln Gly Asp Leu Leu Met
 145 150 155 160
 Val Gly Glu Arg Val Leu Ala Gly Tyr Gly Phe Arg Thr Asp Gln Arg
 165 170 175
 40 Ala His Ala Glu Ile Ala Ala Val Leu Gly Leu Pro Val Val Ser Leu
 180 185 190
 45 Glu Leu Val Asp Pro Arg Phe Tyr His Leu Asp Thr Ala Leu Ala Val
 195 200 205
 Leu Asp Asp His Thr Ile Ala Tyr Tyr Pro Pro Ala Phe Ser Thr Ala
 210 215 220
 50 Ala Gln Glu Gln Leu Ser Ala Leu Phe Pro Asp Ala Ile Val Val Gly
 225 230 235 240
 55 Ser Ala Asp Ala Phe Val Phe Gly Leu Asn Ala Val Ser Asp Gly Leu
 245 250 255

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5 Asn Val Val Leu Pro Val Ala Ala Met Gly Phe Ala Ala Gln Leu Arg
 260 265 270

Ala Ala Gly Phe Glu Pro Val Gly Val Asp Leu Ser Glu Leu Leu Lys
 275 280 285

10 Gly Gly Gly Ser Val Lys Cys Cys Thr Leu Glu Ile His Pro
 290 295 300

<210> 162

15 <211> 906

<212> DNA

<213> Mycobacterium tuberculosis

20

<400> 162
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 25 acgcggcgggt atgcaatgac ccgcgcggcc ttctttgccg tcgcatacgc gatcaacccc 180
 tggatggacg tcaccgcgcc agtcgacgtc caagtcgcgc aagcacagtg ggagcacctc 240
 caccagacct atcttcgggt aggccacagc gtggatctga tcgagcccat ttccgggtta 300
 30 ccggacatgg tgtacaccgc caacggtggg ttcatcgcgc acgacatcgc cgtggtcgcc 360
 cggttccgggt tccccgaacg agctggtgag tctagagcct atgccagctg gatgtcctcg 420
 gtcggatata gcccggtgac caccgccac gtcaacgagg gacagggcga cctgctgatg 480
 35 gttggcgaaa ggggtgttggc gggctacggc ttctgcacag accagcgcgc acacgccgaa 540
 atgcgcgcgg tgcttgggtct gccggtggtc tccctcgagt tggtcgaccc acggttctat 600
 cacctggaca ccgcgtggc cgtgctcgac gaccacacga tcgcctacta cccgccggcg 660
 40 ttcagtacgg cagcgcagga acagttgtcg gcgctgttcc ccgacgcgat tgtggtcggc 720
 agtgccgacg cgttcgtgtt cggactcaac gccgtctctg acggtctgaa cgtagtgttt 780
 ccggtcgcgg ccatgggttt tgcggcgcag ttacgcgcag ccggcttcga gccggtcggg 840
 45 gtcgatctgt ccgagctgct caagggcggc ggttccgtca agtgctgcac gctggagata 900
 caccca 906

50 <210> 163

<211> 652

<212> PRT

55 <213> Mycobacterium tuberculosis

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<400> 163

5	Met	Val	Glu	Ser	Gly	Thr	Gly	Ile	Pro	Leu	Pro	Ala	Ser	Cys	Trp	Ser	1	5	10	15
	Arg	Thr	Arg	Ser	Arg	Arg	Cys	Met	Pro	Lys	Asp	Ser	Ser	Pro	His	Trp	20	25	30	
10	Ile	Trp	His	Ser	Ser	Ala	Ala	Arg	Ala	Ala	Leu	Tyr	Val	Arg	Gly	Lys	35	40	45	
15	Arg	Arg	Pro	Asp	Gly	Gln	Gly	Arg	Arg	Ser	Cys	Ala	Leu	Arg	Asn	Arg	50	55	60	
	Gly	Arg	Thr	Pro	Ala	Thr	Gly	Pro	Gly	Pro	Gly	Gln	Ser	Pro	Ser	Pro	65	70	75	80
20	Val	Gly	Ala	Arg	Gln	Pro	Ala	Leu	Pro	Ser	Arg	Arg	Pro	Leu	Asn	Pro	85	90	95	
25	Ala	Arg	Ser	Arg	Thr	Glu	Val	Val	Met	Ser	Asp	Ala	Arg	Val	Pro	Arg	100	105	110	
	Ile	Pro	Ala	Ala	Leu	Ser	Ala	Pro	Ser	Leu	Asn	Arg	Gly	Val	Gly	Phe	115	120	125	
30	Thr	His	Ala	Gln	Arg	Arg	Arg	Leu	Gly	Leu	Thr	Gly	Arg	Leu	Pro	Ser	130	135	140	
35	Ala	Val	Leu	Thr	Leu	Asp	Gln	Gln	Ala	Glu	Arg	Val	Trp	His	Gln	Leu	145	150	155	160
	Gln	Ser	Leu	Ala	Thr	Glu	Leu	Gly	Arg	Asn	Leu	Leu	Leu	Glu	Gln	Leu	165	170	175	
40	His	Tyr	Arg	His	Glu	Val	Leu	Tyr	Phe	Lys	Val	Leu	Ala	Asp	His	Leu	180	185	190	
45	Pro	Glu	Leu	Met	Pro	Val	Val	Tyr	Thr	Pro	Thr	Val	Gly	Glu	Ala	Ile	195	200	205	
	Gln	Arg	Phe	Ser	Asp	Glu	Tyr	Arg	Gly	Gln	Arg	Gly	Leu	Phe	Leu	Ser	210	215	220	
50	Ile	Asp	Glu	Pro	Asp	Glu	Ile	Glu	Glu	Ala	Phe	Asn	Thr	Leu	Gly	Leu	225	230	235	240
55	Gly	Pro	Glu	Asp	Val	Asp	Leu	Ile	Val	Cys	Thr	Asp	Ala	Glu	Ala	Ile	245	250	255	

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Leu Gly Ile Gly Asp Trp Gly Val Gly Gly Ile Gln Ile Ala Val Gly
 260 265 270
 5
 Lys Leu Ala Leu Tyr Thr Ala Gly Gly Gly Val Asp Pro Arg Arg Cys
 275 280 285
 10
 Leu Ala Val Ser Leu Asp Val Gly Thr Asp Asn Glu Gln Leu Leu Ala
 290 295 300
 Asp Pro Phe Tyr Leu Gly Asn Arg His Ala Arg Arg Arg Gly Arg Glu
 305 310 315 320
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 Tyr Asp Glu Phe Val Ser Arg Tyr Ile Glu Thr Ala Gln Arg Leu Phe
 325 330 335
 Pro Arg Ala Ile Leu His Phe Glu Asp Phe Gly Pro Ala Asn Ala Arg
 340 345 350
 20
 Lys Ile Leu Asp Thr Tyr Gly Thr Asp Tyr Cys Val Phe Asn Asp Asp
 355 360 365
 25
 Met Gln Gly Thr Gly Ala Val Val Leu Ala Ala Val Tyr Ser Gly Leu
 370 375 380
 Lys Val Thr Gly Ile Pro Leu Arg Asp Gln Thr Ile Val Val Phe Gly
 385 390 395 400
 30
 Ala Gly Thr Ala Gly Met Gly Ile Ala Asp Gln Ile Arg Asp Ala Met
 405 410 415
 35
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 420 425 430
 Ile Asp Arg Pro Gly Leu Leu Phe Asp Asp Met Asp Asp Leu Arg Asp
 435 440 445
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 Phe Gln Val Pro Tyr Ala Lys Asn Arg His Gln Leu Gly Val Ala Val
 450 455 460
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 Gly Asp Arg Val Gly Leu Ser Asp Ala Ile Lys Ile Ala Ser Pro Thr
 465 470 475 480
 Ile Leu Leu Gly Cys Ser Thr Val Tyr Gly Ala Phe Thr Lys Glu Val
 485 490 495
 50
 Val Glu Ala Met Thr Ala Ser Cys Lys His Pro Met Ile Phe Pro Leu
 500 505 510
 55
 Ser Asn Pro Thr Ser Arg Met Glu Ala Ile Pro Ala Asp Val Leu Ala
 515 520 525

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5 Trp Ser Asn Gly Arg Ala Leu Leu Ala Thr Gly Ser Pro Val Ala Pro
530 535 540

Val Glu Phe Asp Glu Thr Thr Tyr Val Ile Gly Gln Ala Asn Asn Val
545 550 555 560

10 Leu Ala Phe Pro Gly Ile Gly Leu Gly Val Ile Val Ala Gly Ala Arg
565 570 575

Leu Ile Thr Arg Arg Met Leu His Ala Ala Ala Lys Ala Ile Ala His
580 585 590

15 Gln Ala Asn Pro Thr Asn Pro Gly Asp Ser Leu Leu Pro Asp Val Gln
595 600 605

20 Asn Leu Arg Ala Ile Ser Thr Thr Val Ala Glu Ala Val Tyr Arg Ala
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Ala Val Gln Asp Gly Val Ala Ser Arg Thr His Asp Asp Val Arg Gln
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645 650

30 <210> 164
<211> 1956
<212> DNA
<213> Mycobacterium tuberculosis

35

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50 cggcttccgt cggccgtgct cacgctcgac caacaggccg aacgcgtatg gcatcagttg 480
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55 acgcccaccg ttggcgaggc aatccaacgc ttctccgacg aataccgcgg gcaacgcgga 660

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 25 acggcgctct gcaaacaccc gatgatcttt ccgctgtcca acccgacgtc gcgcatggaa 1560
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 30 ttggcgtttc ccggcatcgg actgggcgct attgtcgtcg gtgcccgggt gataaccagg 1740
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 gactcgtgtg tgccgatgt ccaaaatctg cgggccatct cgacaacggt cgccgaagct 1860
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40 <210> 165

<211> 356

<212> PRT

45 <213> Mycobacterium tuberculosis

<400> 165

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 20 25 30

55 Gly Ile Gly Val Gly Cys His Gly Gly Pro Ser Asp Val Val Gly Arg

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	35		40		45	
5	Ala Gly Pro Asp Arg Ala His Thr Ser Ile Thr Leu Val Ala Tyr Ala	50	55	60		
	Val Pro Glu Pro Gly Trp Ser Ala Val Ile Pro Ala Phe Asn Ala Ser	65	70	75	80	
10	Glu Gln Gly Arg Gly Val Gln Val Ile Thr Ser Tyr Gly Ala Ser Ala	85	90	95		
15	Asp Gln Ser Arg Gly Val Ala Asp Gly Lys Pro Ala Asp Leu Val Asn	100	105	110		
	Phe Ser Val Glu Pro Asp Ile Ala Arg Leu Val Lys Ala Gly Lys Val	115	120	125		
20	Asp Lys Asp Trp Asp Ala Asp Ala Thr Lys Gly Ile Pro Phe Gly Ser	130	135	140		
25	Val Val Thr Phe Val Val Arg Ala Gly Asn Pro Lys Asn Ile Arg Asp	145	150	155	160	
	Trp Asp Asp Leu Leu Arg Pro Gly Ile Glu Val Ile Thr Pro Ser Pro	165	170	175		
30	Leu Ser Ser Gly Ser Ala Lys Trp Asn Leu Leu Ala Pro Tyr Ala Ala	180	185	190		
35	Lys Ser Asp Gly Gly Arg Asn Asn Gln Ala Gly Ile Asp Phe Val Asn	195	200	205		
	Thr Leu Val Asn Glu His Val Lys Leu Arg Pro Gly Ser Gly Arg Glu	210	215	220		
40	Ala Thr Asp Val Phe Val Gln Gly Ser Gly Asp Val Leu Ile Ser Tyr	225	230	235	240	
	Glu Asn Glu Ala Ile Ala Thr Glu Arg Ala Gly Lys Pro Val Gln His	245	250	255		
45	Val Thr Pro Pro Gln Thr Phe Lys Ile Glu Asn Pro Leu Ala Val Val	260	265	270		
50	Ala Thr Ser Thr His Leu Gly Ala Ala Thr Ala Phe Arg Asn Phe Gln	275	280	285		
55	Tyr Thr Val Gln Ala Gln Lys Leu Trp Ala Gln Ala Gly Phe Arg Pro	290	295	300		

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Val Asp Pro Ala Val Ala Ala Asp Phe Ala Asp Leu Phe Pro Val Pro
305 310 315 320

5 Ala Lys Leu Trp Thr Ile Ala Asp Leu Gly Gly Trp Gly Ser Val Asp
325 330 335

10 Pro Gln Leu Phe Asp Lys Ala Thr Gly Ser Ile Thr Lys Ile Tyr Leu
340 345 350

Arg Ala Thr Gly
355

15 <210> 166

<211> 1068

<212> DNA

20 <213> Mycobacterium tuberculosis

<400> 166

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30 gtcgcctacg cgtgccgga acccggtggt agtgcggtga ttcccgcggt caacgcttcc 240

gaacagggcc ggggagtgca ggtgattacc tcatatggcg cgtcgccga ccagtcgcgc 300

ggtgttgccg acggtaaacc ggccgacctg gtgaacttct cggtcgaacc ggacatcgct 360

35 cgcttggtca aggcggcaa ggttgacaag gactgggacg ccgatgccac caagggcac 420

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tgggatgacc tggtgcgcc ggggtattgag gtcacacgc ccagtcgct gagtcgggt 540

tctgccaagt ggaatctgct agccccctac gccgcgaaaa gtgacggtgg ccggaataac 600

40 caagcgggga tcgactttgt caatacattg gtgaatgaac acgtcaaatt gcgccccggg 660

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55 <210> 167

<211> 514

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<212> PRT

<213> Mycobacterium tuberculosis

<400> 167

5

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15

Asn Val Cys Ala Leu Cys Cys Val Val Val Ala Leu Gly Gly Gly Ala
35 40 45

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Leu Trp Trp Cys Val Ala Arg Arg Ser Trp His Ala Pro Arg Leu Gly
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Ser Ile Ser Ala Gly Leu Val Ala Val Gly Met Val Gly Ala Gly Tyr
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25

Gly Leu Ala Val Ala Leu Arg Ser Glu Ala Val Asp Arg His Pro Ile
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30

Thr Val Ala Phe Gly Thr Ser Ala Leu Val Thr Val Thr Pro Ser Glu
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Ser Pro Val Ser Leu Gly Arg Gly Arg Leu Met Phe Arg Ala Thr Val
115 120 125

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Gln Arg Leu Arg Asp Asp Glu Thr Ser Gly Arg Val Val Val Phe Ala
130 135 140

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Arg Ala Leu Asp Phe Gly Glu Leu Met Val Gly Gln Pro Val Gln Phe
145 150 155 160

Arg Ala Arg Ile Ser Arg Pro Ala Arg His Asp Leu Thr Val Ala Val
165 170 175

45

Phe Asn Ala Thr Gly Arg Pro Thr Val Gly Arg Ala Gly Pro Val His
180 185 190

50

Arg Ala Ala His Ile Val Arg His Arg Phe Ala Ala Ala Val Arg Glu
195 200 205

Val Leu Pro Ala Asp Gln Ala Thr Met Leu Pro Ala Leu Val Leu Gly
210 215 220

55

Asp Thr Ser Thr Val Thr Ala Leu Thr Ser Arg Glu Phe Arg Ala Ala

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	Ala	Asn	Leu	Ala	Val	Ala	Ala	Val	Ile	Ala	Pro	Ile	Thr	Val	Leu	Gly
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	Leu	Ile	Arg	Phe	Thr	Gly	Pro	Glu	Val	Trp	Trp	Val	Leu	Arg	Val	Ala
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50	Leu	Pro	Gly	Val	Leu	Leu	Val	Gly	Gly	Ala	Thr	Val	Phe	Thr	Val	Ala
	465					470					475					480
	Gln	Trp	Arg	Trp	Arg	Trp	Phe	Arg	Ala	Ala	Met	Cys	Lys	Thr	Met	Ala
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Val Ala Val Ile Cys Leu Leu Ala Trp Ser Leu Ser Gly Leu Val Gly
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5 Pro Ser

<210> 168

10 <211> 1542

<212> DNA

<213> Mycobacterium tuberculosis

15

<400> 168

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<211> 139

10 <212> PRT

<213> Mycobacterium tuberculosis

15 <400> 169

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Ala Thr Val Tyr Ala Gln Leu Ala Met Gly Asp Ser Trp Arg Ile Gly
35 40 45

25 Val Asp Glu Gln Glu Asn Thr Thr Leu Val Arg Thr Gly Pro Phe Lys
50 55 60

30 Trp Val Arg His Pro Ile Tyr Thr Ala Met Met Ala Phe Gly Leu Gly
65 70 75 80

Leu Leu Leu Val Thr Pro Asn Leu Val Ala Leu Ala Gly Phe Ile Leu
85 90 95

35 Leu Val Ala Thr Leu Glu Val His Val Arg Arg Val Glu Glu Pro Tyr
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40 Leu Leu Arg Thr His Ser Ala Val Tyr Arg Gly Tyr Thr Ala Ser Val
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<211> 417

50 <212> DNA

<213> Mycobacterium tuberculosis

55 <400> 170

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 <211> 161
 15 <212> PRT
 <213> Mycobacterium tuberculosis

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 30 Ser Asp Gly Gly Trp Glu Pro Gly Asn Ser Leu Phe Ile Thr Val Asn
 35 40 45
 Cys Trp Gly Arg Leu Val Thr Gly Val Gly Ala Ala Leu Gly Lys Gly
 50 55 60
 35 Ala Pro Val Ile Val Val Gly His Val Tyr Thr Ser Glu Tyr Glu Asp
 65 70 75 80
 40 Arg Asp Gly Ile Arg Arg Ser Ser Leu Glu Met Arg Ala Thr Ser Val
 85 90 95
 Gly Pro Asp Leu Ser Arg Val Ile Val Arg Ile Glu Lys Pro Ala Tyr
 100 105 110
 45 Thr Gly Pro Ser Ala Gly Asp Leu Pro Ala Ala Thr Gly Thr Gly Ala
 115 120 125
 50 Ala Gly Ala Ala Asp Ala Pro Ala Ser Ala Ala Asp Ser Val Ser Asp
 130 135 140
 Val Val Val Asp Asp Ala Ile Thr Gly His Asn Pro Leu Pro Ile Ser
 145 150 155 160
 55 Ala

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 5 <211> 483
 <212> DNA
 <213> Mycobacterium tuberculosis
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 20 25 30
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 35 40 45
 Asp Val His Ala Val Val Val Thr Gly Ala Gly Lys Ala Phe Cys Ala
 50 50 55 60
 Gly Ala Asp Leu Ser Ala Leu Gly Ala Gly Val Gly Asp Pro Ala Glu
 65 70 75 80
 55 Pro Arg Leu Leu Arg Leu Tyr Asp Gly Phe Met Ala Val Ser Ser Cys
 85 90 95

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5 Asn Leu Pro Thr Ile Ala Ala Val Asn Gly Ala Ala Val Gly Ala Gly
 100 105 110
 Leu Asn Leu Ala Leu Ala Ala Asp Val Arg Ile Ala Gly Pro Ala Ala
 115 120 125
 10 Leu Phe Asp Ala Arg Phe Gln Lys Leu Gly Leu His Pro Gly Gly Gly
 130 135 140
 15 Ala Thr Trp Met Leu Gln Arg Ala Val Gly Pro Gln Val Ala Arg Ala
 145 150 155 160
 Ala Leu Leu Phe Gly Met Cys Phe Asp Ala Glu Ser Ala Val Arg His
 165 170 175
 20 Gly Leu Ala Leu Met Val Ala Asp Asp Pro Val Thr Ala Ala Leu Glu
 180 185 190
 25 Leu Ala Ala Gly Pro Ala Ala Ala Pro Arg Glu Val Val Leu Ala Ser
 195 200 205
 Lys Ala Thr Met Arg Ala Thr Ala Ser Pro Gly Ser Leu Asp Leu Glu
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 30 Gln His Glu Leu Ala Lys Arg Leu Glu Leu Gly Pro Gln Ala Lys Ser
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 245 250 255
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 40 <212> DNA
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 45 <400> 174
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 55 gtgcgcateg ccggaccggc cgcattgttc gacgcccgt tccaaaagct gggactgcat 420

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<210> 175
 15 <211> 547
 <212> PRT
 <213> Mycobacterium tuberculosis

<400> 175
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 30 Ala Ala Thr Ala Ala Arg Tyr Gly His Arg Glu Ala Leu Val Asp Met
 35 40 45
 Val Ala Arg Arg Arg Phe Asn Tyr Ser Glu Leu Leu Thr Asp Val His
 50 55 60
 35 Arg Leu Ala Thr Gly Leu Val Arg Ala Gly Ile Gly Pro Gly Asp Arg
 65 70 75 80
 40 Val Gly Ile Trp Ala Pro Asn Arg Trp Glu Trp Val Leu Val Gln Tyr
 85 90 95
 Ala Thr Ala Glu Ile Gly Ala Ile Leu Val Thr Ile Asn Pro Ala Tyr
 100 105 110
 45 Arg Val Arg Glu Val Glu Tyr Ala Leu Arg Gln Ser Gly Val Ala Met
 115 120 125
 50 Val Ile Ala Val Ala Ser Phe Lys Asp Ala Asp Tyr Ala Ala Met Leu
 130 135 140
 Ala Glu Val Gly Pro Arg Cys Pro Asp Leu Ala Asp Val Ile Leu Leu
 145 150 155 160
 55 Glu Ser Asp Arg Trp Asp Ala Leu Ala Gly Ala Glu Pro Asp Leu Pro

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40	Asp	Ser	Val	Asp	Arg	Arg	Val	Gly	Thr	Val	Gly	Arg	Val	Gly	Pro	His		
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	Leu	Glu	Ile	Lys	Val	Val	Asp	Pro	Ala	Thr	Gly	Glu	Thr	Val	Pro	Arg		
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55				420						425				430				

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Arg Ile Ala Gly Arg Ile Lys Asp Leu Val Val Arg Gly Gly Glu Asn
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 5
 Ile Ser Pro Arg Glu Ile Glu Glu Leu Leu His Thr His Pro Asp Ile
 450 455 460
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 Val Asp Gly His Val Ile Gly Val Pro Asp Ala Lys Tyr Gly Glu Glu
 465 470 475 480
 Leu Met Ala Val Val Lys Leu Arg Asn Asp Ala Pro Glu Leu Thr Ile
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 Glu Arg Leu Arg Glu Tyr Cys Met Gly Arg Ile Ala Arg Phe Lys Ile
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 Pro Arg Tyr Leu Trp Ile Val Asp Glu Phe Pro Met Thr Val Thr Gly
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 <212> DNA
 <213> Mycobacterium tuberculosis
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<210> 177

<211> 463

<212> PRT

<213> Mycobacterium tuberculosis

<400> 177

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35 40 45

Pro Arg Leu Arg Arg Asp Pro Thr Gly Gly Gly Ser Thr Pro Ser Ile
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Val Leu Ser Arg Ser Thr Asp Arg Ser Lys Asp Gly His Arg Ile Val
65 70 75 80

55 Pro Ala Gly Ala Arg Lys Ser Gly Val Arg Ala Ser Thr Gly Arg Leu

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10	Gly Ser Ser Glu Cys Leu Leu His Arg Cys Arg Thr Pro Pro Val Pro 130 135 140		
15	Ser His Ser Val Glu Leu Leu Val Ala Ala Asn Pro Ala Glu Asp Ser 145 150 155 160		
	Arg Leu Pro Tyr Leu Ile Arg Leu Pro Val Gly Ala Gly Leu Val Phe 165 170 175		
20	Ala Thr Ser Asp Val Trp Pro Arg Thr Lys Ala Leu Tyr Cys His Arg 180 185 190		
25	Leu Asp Ile Ala Asp Trp Pro Ala Asp Pro Val Val Val Asp Arg Val 195 200 205		
	Glu Leu Arg Ser Cys Ser Arg Arg Gly Ala Ala Ile Asp Val Val Ala 210 215 220		
30	Ala Arg Ala Arg Glu Asn Arg Ser Gln Leu Val His Thr Met Ala Arg 225 230 235 240		
35	Gly Arg Gln Val Val Phe Trp Gln Ser Pro Lys Thr Arg Lys Gln Ser 245 250 255		
	Arg Pro Gly Val Arg Thr Pro Thr Ala Arg Ala Ala Gly Ile Pro Glu 260 265 270		
40	Leu His Ile Val Val Asp Ala His Glu Arg Tyr Pro Tyr Thr Phe Ala 275 280 285		
45	Asp Lys Pro Ala Lys Thr Thr Arg Glu Ala Leu Pro Cys Gly Asp Tyr 290 295 300		
	Gly Leu Lys Val Ala Gly Gln Leu Val Ala Ala Val Glu Arg Lys Ala 305 310 315 320		
50	Leu Ala Asp Leu Thr Ser Gly Val Leu Asn Gly Asn Leu Lys Tyr Gln 325 330 335		
55	Leu Thr Glu Leu Ala Ala Leu Pro Arg Ala Ala Val Val Val Glu Asp 340 345 350		

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	Arg Tyr Ser Glu Ile Phe Ala His Ser Phe Ala Arg Pro Thr Ala Ile	
	355 360 365	
5	Ala Asp Gly Leu Ala Glu Leu Gln Ile Gly Phe Pro Asn Val Pro Ile	
	370 375 380	
	Val Phe Cys Gln Thr Arg Lys Leu Ala Gln Glu Tyr Thr Tyr Arg Tyr	
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	Leu Ala Ala Ala Leu Thr Trp Phe Val Asp Asp Ala Asp Ala Thr Thr	
	405 410 415	
15	Val Phe Glu Pro Ala Ala Ala Glu Pro Glu Pro Ser Ser Ala Glu Leu	
	420 425 430	
	Arg Ala Trp Ala Lys Ser Val Gly Leu Pro Val Ser Asp Arg Gly Arg	
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30	<213> Mycobacterium tuberculosis	
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<211> 134

<212> PRT

<213> Mycobacterium tuberculosis

<400> 179

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Leu Ile Ala His Ala Ala Val Glu Thr Tyr Ser Val Leu Thr Arg Leu
 35 40 45

Pro Pro Pro His Arg Ile Ala Pro Val Ala Val His Ala Tyr Leu Ala
 50 55 60

Asp Ile Thr Ser Ser Asn Tyr Leu Ala Leu Asp Ala Cys Ser Tyr Arg
 65 70 75 80

Gly Leu Thr Asp His Leu Ala Glu His Asp Val Thr Gly Gly Ala Thr
 85 90 95

Tyr Asp Ala Leu Val Gly Phe Thr Ala Lys Ala Ala Gly Ala Lys Leu
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Glu Val Glu Leu Val Thr

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 10 <213> Mycobacterium tuberculosis

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 <212> PRT
 30 <213> Mycobacterium tuberculosis

 <400> 181
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 35 40 45
 45 Pro Ala Val Glu Ala Met Asp Gln Ile Thr Leu Ser Val Arg Asp Tyr
 50 55 60

 Arg Asp Gly Asp Leu Asp Gly Ala Trp Tyr Ala Ile Ala Ala Thr Asp
 65 70 75 80
 50
 Asp Ala Arg Val Asn Val Ala Val Val Ala Glu Ala Glu Arg Arg Arg
 85 90 95
 55 Ile Phe Cys Val Arg Ala Asp Ile Ala Val Glu Gly Thr Ala Val Thr
 100 105 110

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5 Pro Ala Ser Phe Ser Tyr Ala Gly Leu Ser Val Gly Val Leu Ala Gly
 115 120 125
 Gly Glu His Arg Arg Ser Ala Ala Ile Arg Ser Ala Ile Arg Glu Ala
 130 135 140
 10 Leu Gln Gln Gly Val Ile Thr Ala Gln Ser Ser Asp Val Leu Ser Gly
 145 150 155 160
 Gly Val Ala Leu Val Gly Gly Gly Pro Gly Asp Pro Glu Leu Ile Thr
 15 165 170 175
 Val Arg Gly Arg Arg Leu Leu Ala Gln Ala Asp Val Val Val Ala Asp
 180 185 190
 20 Arg Leu Ala Pro Pro Glu Leu Leu Ala Glu Leu Pro Pro His Val Glu
 195 200 205
 Val Ile Asp Ala Ala Lys Ile Pro Tyr Gly Arg Ala Met Ala Gln Asp
 25 210 215 220
 Ala Ile Asn Ala Val Leu Ile Glu Arg Ala Arg Ser Gly Asn Phe Val
 225 230 235 240
 30 Val Arg Leu Lys Gly Gly Asp Pro Phe Val Phe Ala Arg Gly Tyr Glu
 245 250 255
 Glu Val Leu Ala Cys Ala His Ala Gly Ile Pro Val Thr Val Val Pro
 35 260 265 270
 Gly Val Thr Ser Ala Ile Ala Val Pro Ala Met Ala Gly Val Pro Val
 275 280 285
 40 Thr His Arg Ala Met Thr His Glu Phe Val Val Val Ser Gly His Leu
 290 295 300
 Ala Pro Gly His Pro Glu Ser Leu Val Asn Trp Asp Ala Leu Ala Ala
 45 305 310 315 320
 Leu Thr Gly Thr Ile Val Leu Leu Met Ala Val Glu Arg Ile Glu Leu
 325 330 335
 50 Phe Val Asp Val Leu Leu Lys Gly Gly Arg Thr Ala Asp Thr Pro Val
 340 345 350
 Leu Val Val Gln His Gly Thr Thr Ala Ala Gln Gln Thr Leu Arg Ala
 55 355 360 365

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Thr Leu Ala Asp Thr Pro Glu Lys Val Arg Ala Ala Gly Ile Arg Pro
370 375 380

5 Pro Ala Ile Ile Val Ile Gly Ala Val Val Gly Leu Ser Gly Val Arg
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Gly Leu Asn Asn Ser
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<210> 182

<211> 1215

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<212> DNA

<213> Mycobacterium tuberculosis

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<400> 182

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 <211> 591
 5 <212> PRT
 <213> Mycobacterium tuberculosis

 10 <400> 183
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 20 25 30
 Phe Tyr Trp Asp Asp Leu Ile Leu Val Gly Arg Ala Gly Thr Gly Gly
 35 40 45
 20 Leu Leu Ser Pro Ser Tyr Leu Phe Asp Asp His Asp Gly His Val Met
 50 55 60
 25 Pro Gly Ala Phe Leu Val Ala Gly Ala Ile Ile Arg Val Ala Pro Leu
 65 70 75 80
 Val Trp Thr Gly Pro Ala Ile Ser Leu Val Val Leu Gln Leu Leu Glu
 85 90 95
 30 Ser Leu Ala Leu Leu Arg Ala Leu Tyr Val Ile Ser Ser Trp Arg Pro
 100 105 110
 35 Val Leu Leu Ile Pro Leu Thr Phe Ala Leu Phe Thr Pro Leu Ala Val
 115 120 125
 Pro Gly Phe Ala Trp Trp Ala Ala Ala Leu Asn Ser Leu Pro Met Leu
 130 135 140
 40 Ala Ala Leu Ala Trp Val Cys Ala Asp Ala Ile Leu Leu Val Arg Thr
 145 150 155 160
 45 Gly Asn His Arg Tyr Ala Val Thr Gly Val Leu Val Tyr Leu Gly Gly
 165 170 175
 Leu Leu Phe Phe Glu Lys Ala Ala Val Ile Pro Phe Val Ser Phe Ala
 180 185 190
 50 Val Ala Ala Leu Gln Cys His Val Arg Gly Asp Arg Ser Ala Leu Ala
 195 200 205
 55 Thr Val Trp Arg Ala Gly Val Arg Leu Trp Thr Pro Ser Leu Ala Leu
 210 215 220

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 Asp Gly Pro Leu Leu Pro Gly Asp Trp Thr Val Glu Leu Asn Tyr Leu
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 10 Ala Asn Ser Asp Gly Ser Met Ala Leu Ala Leu Ser Asp Gly Pro Glu
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 Arg Lys Val Pro Val His Pro Gly Leu Asn Arg Val Tyr Ala Arg Leu
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<211> 498

<212> PRT

<213> Mycobacterium tuberculosis

<400> 185

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 35 40 45

Leu Glu Gly Val Gln Leu Asp Gly Ile Ser Leu Pro Ile Ala Leu Gly
 50 55 60

Leu Leu Ile Met Met Tyr Pro Val Leu Ala Lys Val Arg Tyr Asp Arg
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Leu Asp Thr Val Thr Gly Asp Arg Lys Leu Leu Leu Ser Ser Leu Leu
 85 90 95

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15	Gly	Asp	Arg	Glu	Ala	Ala	Ala	Val	Leu	Val	Ala	Leu	Asn	Ser	Ile	Phe
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70	Thr	Ser	Gly	Gln	Ala	Leu	Ala	Gly	Val	Val	Gly	Pro	Leu	Ile	Glu	Val
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75	Pro	Val	Leu	Val	Gly	Leu	Val	Tyr	Val	Ser	Leu	Ala	Leu	Arg	Asn	Arg
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 Leu Thr His Leu Ala Gly Asp Arg Ile Glu Val Arg Ser Ala Gly Thr
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 405 410 415
 15 Met Gly Ile Asp Ile Thr Ala Asn Ala Pro Thr Leu Leu Thr Gly Gly
 420 425 430
 Gln Val Gln Ser Ser Asp Val Val Ile Thr Met Gly Cys Gly Asp Ala
 435 440 445
 20 Cys Pro Tyr Phe Pro Gly Val Ser Tyr Arg Asn Trp Lys Leu Pro Asp
 450 455 460
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 40 <400> 186
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<212> PRT

35 <213> Mycobacterium tuberculosis

<400> 187

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 35 40 45

50 Thr Val Arg Lys Trp Val Arg Gln Ala Gln Val Asp Ala Gly Ala Arg
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Pro Gly Thr Thr Thr Glu Glu Ser Ala Glu Leu Lys Arg Leu Arg Arg
 65 70 75 80

55 Asp Asn Ala Glu Leu Arg Arg Ala Asn Ala Ile Leu Lys Thr Ala Ser

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Ala Phe Phe Ala Ala Glu Leu Asp Arg Pro Ala Arg
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20 25 30

Asp Ile Arg Ala Phe Leu Arg Arg Phe Val Val Tyr Pro Ser Asp His
35 40 45

45

Glu Leu Ile Ala His Thr Leu Trp Ile Ala His Cys Trp Phe Met Glu
50 55 60

50

Ala Trp Asp Ser Thr Pro Arg Ile Ala Phe Leu Ser Pro Glu Pro Gly
65 70 75 80

Ser Gly Lys Ser Arg Ala Leu Glu Val Thr Glu Pro Leu Val Pro Arg
85 90 95

55

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	Pro	Val	His	Ala	Ile	Asn	Cys	Thr	Pro	Ala	Tyr	Leu	Phe	Arg	Arg	Val	100	105	110
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	Asn	Ala	Gly	His	Arg	Lys	Gly	Ala	Val	Ala	Gly	Arg	Cys	Val	Ile	Arg	145	150	155
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20	Leu	Ala	Gly	Leu	Asp	Asp	Leu	Pro	Asp	Thr	Ile	Met	Ser	Arg	Ser	Ile	180	185	190
	Val	Val	Arg	Met	Arg	Arg	Arg	Ala	Pro	Thr	Glu	Pro	Val	Glu	Pro	Trp	195	200	205
25	Arg	Pro	Arg	Val	Asn	Gly	Pro	Glu	Ala	Glu	Lys	Leu	His	Asp	Arg	Leu	210	215	220
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	Met	Pro	Asp	Gly	Val	Thr	Asp	Arg	Arg	Ala	Asp	Val	Trp	Glu	Ser	Leu	245	250	255
35	Val	Ala	Val	Ala	Asp	Thr	Ala	Gly	Gly	His	Trp	Pro	Lys	Thr	Ala	Arg	260	265	270
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45	Asp	Arg	Met	Arg	Thr	Ser	Asp	Ile	Leu	Thr	Gly	Leu	Asn	Arg	Met	Glu	305	310	315
50	Glu	Gly	Pro	Trp	Gly	Ser	Ile	Arg	Arg	Gly	Asp	Pro	Leu	Asp	Ala	Arg	325	330	335
	Gly	Leu	Ala	Thr	Arg	Leu	Gly	Arg	Tyr	Gly	Ile	Gly	Pro	Lys	Phe	Gln	340	345	350
55	His	Ser	Gly	Gly	Glu	Pro	Pro	Tyr	Lys	Gly	Tyr	Ser	Arg	Thr	Gln	Phe	355	360	365

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Glu Asp Ala Trp Ser Arg Tyr Leu Ser Ala Asp Asp Glu Thr Pro Glu
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 Asn Gly Asp Ala Pro Leu Cys Ser Gly Pro Gly Cys Pro Asn Lys Leu
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<210> 191

<211> 429

<212> PRT

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<213> Mycobacterium tuberculosis

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<400> 191

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Ala Val Val Val Leu Pro Val Ile Thr Ser His Asp Ile Phe Tyr Ser
35 40 45

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His Asp Thr Gly Ile Asp Trp Asp Val Ile Phe Leu Leu Val Gly Met
50 55 60

Met Ile Ile Val Gly Val Leu Arg Gln Thr Gly Val Phe Glu Tyr Thr
65 70 75 80

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Ala Ile Trp Ala Ala Lys Arg Ala Arg Gly Ser Pro Leu Arg Ile Met
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Ile Leu Leu Val Leu Val Ser Ala Leu Ala Ser Ala Leu Leu Asp Asn
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Val Thr Thr Val Leu Leu Ile Ala Pro Val Thr Leu Leu Val Cys Asp
115 120 125

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Ala Ile Tyr Leu Trp Leu Arg Tyr Phe Val Leu Leu His
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<213> Mycobacterium tuberculosis

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<211> 237

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<213> Mycobacterium tuberculosis

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Ala Val Leu Leu Pro Met Pro Val Ser Leu Arg Pro Val Ala Glu Pro
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Tyr Val Leu Val Gly Asp Ile Ala Ile Ile Gly Phe Phe Phe Val Gly
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Gly Thr Val Phe Phe Glu Lys Gln Glu Arg Thr Ile Gly Ala Ile Val
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Ser Thr Pro Leu Arg Phe Trp Glu Tyr Leu Ala Ala Lys Leu Thr Val
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Leu Leu Ala Ile Ser Leu Phe Val Ala Val Val Val Ala Thr Ile Val
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His Gly Leu Gly Tyr His Leu Leu Pro Leu Val Ala Gly Ile Val Leu
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Gly Thr Leu Leu Met Leu Leu Val Gly Phe Ser Ser Ser Leu Pro Phe
130 135 140

Ala Ser Val Thr Asp Trp Phe Leu Ala Ala Val Ile Pro Leu Ala Ile
145 150 155 160

Met Leu Ala Pro Pro Val Val His Tyr Ser Gly Leu Trp Pro Asn Pro
165 170 175

Val Leu Tyr Leu Ile Pro Thr Gln Gly Pro Leu Leu Leu Leu Gly Ala
180 185 190

Ala Phe Asp Gln Val Ser Leu Ala Pro Trp Gln Val Gly Tyr Ala Val
195 200 205

Val Tyr Pro Ile Val Cys Ala Ala Gly Leu Cys Arg Ala Ala Lys Ala
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<400> 195
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35 40 45
55 Glu Glu Ile Phe Leu Val Leu Ser Val Ala Gly Leu Ala Ala Tyr Ser
50 55 60

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 Val Ala Ser Ala Leu Met Val Asp Tyr Val Leu Thr Val Ala Val Ser
 115 120 125
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 Ile Ser Ser Ala Ala Ser Asn Ile Gly Ser Val Ser Pro Phe Val Tyr
 130 135 140
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 Glu His Lys Val Leu Phe Ala Val Gly Ala Ile Val Leu Ile Met Ala
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 Thr Tyr Ala Phe Ile Ala Gly Ile Gly Thr Met Leu Val Trp Gly Leu
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 Phe Leu Val Ala Arg Ser Phe Ser Ser Gly Cys Ala Ala Leu Thr Gly
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<211> 549

<212> PRT

<213> Mycobacterium tuberculosis

<400> 197

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Asp Gly Val Gln Leu Val Ala Asp His Tyr Ala Pro Ala Thr Ser Gln
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Phe Ser Leu Val Phe Ala Arg Ile Tyr Ala Ala Arg Gly Tyr His Val
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 His Leu Pro Asp Trp Pro Pro Ala Thr Thr Glu Arg Ala Trp Tyr Leu
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 25 Lys Gly Arg Ser Arg Asn Val Ser Asp Gly Tyr Arg Arg Leu Gly Asp
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50 <213> Mycobacterium tuberculosis

<400> 199

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45 <212> DNA

<213> Mycobacterium tuberculosis

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<213> Mycobacterium tuberculosis

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<400> 201

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<213> Mycobacterium tuberculosis

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Leu Gly Leu Ala Leu Ala Met Leu Leu Asp Arg Pro Leu Arg Gly Arg
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20	gtgatcaacg	tgatgaccog	gggcggggcg	gagggcaccg	gcaccaccac	catggtgtac	780
	cagggtgtatg	tggagacggt	ccgcaatttc	cgggcgggtt	atggcgccac	ggtggccacg	840
	atcatgttcc	tggtgtgtgt	ggccgtgacg	tactaccagg	tgccgggtgat	ggatcggggg	900
	cagccggcag						909

<210> 205

<211> 695

<212> PRT

<213> Mycobacterium tuberculosis

<400> 205

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Arg Cys Gly Ile Ala Pro Ala Thr Ser Gln Arg Ser Leu Ala Thr Pro
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Pro Thr Ile Ser Val Pro Ser Gly Glu Gly Arg Cys Arg Cys His Val
35 40 45

45 Ala Arg Gly Ala Gly Arg Asp Pro Arg Arg Arg Leu Arg Arg Arg Arg
50 55 60

Trp Cys Gly Arg Cys Gly Tyr His Ser His Leu Thr Gly Gly Glu Phe
65 70 75 80

50
Asp Val Asn Arg Leu Cys Gln Gln Arg Ser Arg Glu Arg Ser Cys Gln
85 90 95

55 Leu Val Ala Val Pro Ala Asp Pro Arg Pro Lys Arg Gln Arg Ile Thr
 100 105 110

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Asp Val Leu Thr Leu Ala Leu Val Gly Phe Leu Gly Gly Leu Ile Thr
 115 120 125
 5
 Gly Ile Ser Pro Cys Ile Leu Pro Val Leu Pro Val Ile Phe Phe Ser
 130 135 140
 10
 Gly Ala Gln Ser Val Asp Ala Ala Gln Val Ala Lys Pro Glu Gly Ala
 145 150 155 160
 Val Ala Val Arg Arg Lys Arg Ala Leu Ser Ala Thr Leu Arg Pro Tyr
 165 170 175
 15
 Arg Val Ile Gly Gly Leu Val Leu Ser Phe Gly Met Val Thr Leu Leu
 180 185 190
 20
 Gly Ser Ala Leu Leu Ser Val Leu His Leu Pro Gln Asp Ala Ile Arg
 195 200 205
 Trp Ala Ala Leu Val Ala Leu Val Ala Ile Gly Ala Gly Leu Ile Phe
 210 215 220
 25
 Pro Arg Phe Glu Gln Leu Leu Glu Lys Pro Phe Ser Arg Ile Pro Gln
 225 230 235 240
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 Lys Gln Ile Val Thr Arg Ser Asn Gly Phe Gly Leu Gly Leu Ala Leu
 245 250 255
 Gly Val Leu Tyr Val Pro Cys Ala Gly Pro Ile Leu Ala Ala Ile Val
 260 265 270
 35
 Val Ala Gly Ala Thr Ala Thr Ile Gly Leu Gly Thr Val Val Leu Thr
 275 280 285
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 Ala Thr Phe Ala Leu Gly Ala Ala Leu Pro Leu Leu Phe Phe Ala Leu
 290 295 300
 Ala Gly Gln Arg Ile Ala Glu Arg Val Gly Ala Phe Arg Arg Arg Gln
 305 310 315 320
 45
 Arg Glu Ile Arg Ile Ala Thr Gly Ser Val Thr Ile Leu Leu Ala Val
 325 330 335
 50
 Ala Leu Val Phe Asp Leu Pro Ala Ala Leu Gln Arg Ala Ile Pro Asp
 340 345 350
 Tyr Thr Ala Ser Leu Gln Gln Gln Ile Ser Thr Gly Thr Glu Ile Arg
 355 360 365
 55
 Glu Gln Leu Asn Leu Gly Gly Ile Val Asn Ala Gln Asn Ala Gln Leu

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	370		375		380												
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	Pro	Asp	Leu	Lys	Gly	Ile	Thr	Gly	Trp	Leu	Asn	Thr	Pro	Gly	Asn	Lys	
					405					410					415		
10	Pro	Ile	Asp	Leu	Lys	Ser	Leu	Arg	Gly	Lys	Val	Val	Leu	Ile	Asp	Phe	
				420					425					430			
	Trp	Ala	Tyr	Ser	Cys	Ile	Asn	Cys	Gln	Arg	Ala	Ile	Pro	His	Val	Val	
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	Gly	Trp	Tyr	Gln	Ala	Tyr	Lys	Asp	Ser	Gly	Leu	Ala	Val	Ile	Gly	Val	
		450					455					460					
20	His	Thr	Pro	Glu	Tyr	Ala	Phe	Glu	Lys	Val	Pro	Gly	Asn	Val	Ala	Lys	
	465					470					475					480	
	Gly	Ala	Ala	Asn	Leu	Gly	Ile	Ser	Tyr	Pro	Ile	Ala	Leu	Asp	Asn	Asn	
25				485						490					495		
	Tyr	Ala	Thr	Trp	Thr	Asn	Tyr	Arg	Asn	Arg	Tyr	Trp	Pro	Ala	Glu	Tyr	
				500					505					510			
30	Leu	Ile	Asp	Ala	Thr	Gly	Thr	Val	Arg	His	Ile	Lys	Phe	Gly	Glu	Gly	
			515					520					525				
	Asp	Tyr	Asn	Val	Thr	Glu	Thr	Leu	Val	Arg	Gln	Leu	Leu	Asn	Asp	Ala	
35		530					535					540					
	Lys	Pro	Gly	Val	Lys	Leu	Pro	Gln	Pro	Ser	Ser	Thr	Thr	Thr	Pro	Asp	
	545					550					555					560	
40	Leu	Thr	Pro	Arg	Ala	Ala	Leu	Thr	Pro	Glu	Thr	Tyr	Phe	Gly	Val	Gly	
					565					570					575		
	Lys	Val	Val	Asn	Tyr	Gly	Gly	Gly	Gly	Ala	Tyr	Asp	Glu	Gly	Ser	Ala	
45				580					585					590			
	Val	Phe	Asp	Tyr	Pro	Pro	Ser	Leu	Ala	Ala	Asn	Ser	Phe	Ala	Leu	Arg	
			595					600					605				
50	Gly	Arg	Trp	Ala	Leu	Asp	Tyr	Gln	Gly	Ala	Thr	Ser	Asp	Gly	Asn	Asp	
		610					615					620					
	Ala	Ala	Ile	Lys	Leu	Asn	Tyr	His	Ala	Lys	Asp	Val	Tyr	Ile	Val	Val	
55						630					635					640	
	Gly	Gly	Thr	Gly	Thr	Leu	Thr	Val	Val	Arg	Asp	Gly	Lys	Pro	Ala	Thr	

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						645																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											

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ccagatctca aaggcatcac cggttggtc aacacgccc gcaacaagcc gatcgacctg 1260
 aaatcattgc gtggcaaggt ggtgctgatt gacttttggg cctactcctg cattaactgc 1320
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<210> 207

<211> 287

<212> PRT

<213> Mycobacterium tuberculosis

<400> 207

Val Asn Glu Ala Leu Ile Gly Leu Ala Phe Ala Ala Gly Leu Val Ala
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Ala Leu Asn Pro Cys Gly Phe Ala Met Leu Pro Ala Tyr Leu Leu Leu
 20 25 30

Val Val Tyr Gly Gln Asp Ser Ala Gly Arg Thr Gly Pro Leu Ser Ala
 35 40 45

Val Gly Arg Ala Ala Ala Ala Thr Val Gly Met Ala Leu Gly Phe Leu
 50 55 60

Thr Val Phe Gly Ile Phe Gly Ala Leu Thr Ile Ser Ala Ala Thr Ala
 65 70 75 80

Val Gln Arg Tyr Leu Pro Tyr Ala Thr Val Leu Ile Gly Leu Ala Leu
 85 90 95

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Ile Ala Leu Gly Gly Trp Leu Leu Leu Gly Arg Gly Leu Thr Ala Leu
100 105 110

5 Thr Pro Arg Ser Leu Gly Val Arg Trp Ala Pro Thr Val Arg Leu Gly
115 120 125

10 Ser Met Tyr Gly Tyr Gly Ile Ser Tyr Ala Val Ala Ser Leu Ser Cys
130 135 140

15 Thr Ile Gly Pro Phe Leu Ala Val Thr Gly Ala Gly Leu Arg Gly Gly
145 150 155 160

Ser Val Val Gly Ser Val Ala Ile Tyr Leu Ala Tyr Val Ala Gly Leu
165 170 175

20 Thr Leu Val Val Gly Val Leu Ala Val Ala Ala Ala Thr Ala Ser Ser
180 185 190

Ala Leu Ala Asp Arg Leu Arg Arg Ile Leu Pro Phe Val Asn Arg Ile
195 200 205

25 Ser Gly Ala Leu Leu Val Val Val Gly Leu Tyr Val Gly Tyr Tyr Gly
210 215 220

30 Leu Tyr Glu Leu Arg Leu Ile Ala Gly Val Gly Ala Asn Pro Gln Asp
225 230 235 240

Ala Val Ile Ala Ala Ala Gly Arg Leu Gln Gly Ala Leu Ala Gly Trp
245 250 255

35 Val Asn Gln His Gly Ala Trp Pro Trp Ala Val Leu Leu Val Val Leu
260 265 270

40 Val Val Gly Ala Phe Ala Gly Thr Trp Phe Arg Arg Val Arg Arg
275 280 285

<210> 208

<211> 861

45 <212> DNA

<213> Mycobacterium tuberculosis

50 <400> 208

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ggccggacgg ggccgcttag cgcagtgggc cgagcggcag ccgccacggt cgggatggcg 180

55 ctggggtttct tgacggtgtt cggcattctt ggagccctga ccatttcgc ggccacggcg 240

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gtgcagcgat acctgcccta tgccacgggtg ctgacgggtc tggcgctcat cggcctcggc 300
 5 gggtaggtgc tggtaggacg agggctgacg gcgttgacgc cccgatccct cggcgtgcgt 360
 tgggtccaa cggtaggct gggttccatg tatggctacg gcatcagcta tggggttget 420
 tcgctgtcat gcaccatcgg gccgtttctc gcggttaccg gggcaggcct gcggggcggt 480
 10 tcggtcgctg ggagcgtagc gatctatctg gcttatgtcg cgggcctgac cctcgttgtc 540
 ggcgtgcttg ccgtcgcggc cgcgaccgag agctcggcgc tggccgaccg cctacggcga 600
 atcttgccgt tcgtcaaccg gatcagtggc gcgtgctggt tggtaggtcg gctgtacgtg 660
 15 ggttactacg gtctctacga gctgcgcctg attgccggtg tcggggcgaa tccccaggat 720
 gcggtgattg ccgcggccgg ccgcctgcaa ggtgccctgg ctggctgggt taaccagcac 780
 ggtgcatggc cttgggcggg gttgctggtt gtgctggtgg tcggtgcctt cgcgggtacc 840
 20 tggtttcggc gggtagggcg c 861

<210> 209
 <211> 413
 25 <212> PRT
 <213> Mycobacterium tuberculosis

30 <400> 209

Met Leu Thr Val Glu Asp Trp Ala Glu Ile Arg Arg Leu His Arg Ala
 1 5 10 15

35 Glu Gly Leu Pro Ile Lys Met Ile Ala Arg Val Leu Gly Ile Ser Lys
 20 25 30

Asn Thr Val Lys Ser Ala Leu Glu Ser Asn Gln Gln Pro Lys Tyr Glu
 35 40 45

40 Arg Ala Pro Gln Gly Ser Ile Val Asp Ala Val Glu Pro Arg Ile Arg
 50 55 60

45 Glu Leu Leu Gln Ala Tyr Pro Thr Met Pro Ala Thr Val Ile Ala Glu
 65 70 75 80

Arg Ile Gly Trp Glu Arg Ser Ile Arg Val Leu Ser Ala Arg Val Ala
 85 90 95

50 Glu Leu Arg Pro Val Tyr Leu Pro Pro Asp Pro Ala Ser Arg Thr Thr
 100 105 110

55 Tyr Val Ala Gly Glu Ile Ala Gln Cys Asp Phe Trp Phe Pro Pro Ile
 115 120 125

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	Glu	Leu	Pro	Val	Gly	Phe	Gly	Gln	Thr	Arg	Thr	Ala	Lys	Gln	Leu	Pro
	130						135					140				
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	Val	Leu	Thr	Met	Val	Cys	Ala	Tyr	Ser	Arg	Trp	Leu	Leu	Ala	Met	Leu
	145					150					155					160
10	Leu	Pro	Ser	Arg	Cys	Ala	Glu	Asp	Leu	Phe	Ala	Gly	Trp	Trp	Arg	Leu
					165					170					175	
	Ile	Glu	Ala	Leu	Gly	Ala	Val	Pro	Arg	Val	Leu	Val	Trp	Asp	Gly	Glu
				180					185					190		
15																
	Gly	Ala	Ile	Gly	Arg	Trp	Arg	Gly	Gly	Arg	Ser	Glu	Leu	Thr	Thr	Glu
			195					200					205			
20	Cys	Gln	Ala	Phe	Arg	Gly	Thr	Leu	Ala	Ala	Lys	Val	Leu	Ile	Cys	Arg
	210						215					220				
	Pro	Ala	Asp	Pro	Glu	Ala	Lys	Gly	Leu	Ile	Glu	Arg	Ala	His	Asp	Tyr
	225					230					235					240
25	Leu	Glu	Arg	Ser	Phe	Leu	Pro	Gly	Arg	Val	Phe	Ala	Ser	Pro	Ala	Asp
					245					250					255	
	Phe	Asn	Ala	Gln	Leu	Gly	Ala	Trp	Leu	Ala	Leu	Val	Asn	Thr	Arg	Thr
				260					265					270		
30																
	Arg	Arg	Ala	Leu	Gly	Cys	Ala	Pro	Thr	Asp	Arg	Ile	Gly	Ala	Asp	Arg
			275					280					285			
35	Ala	Ala	Met	Leu	Ser	Leu	Pro	Pro	Val	Ala	Pro	Ala	Thr	Gly	Trp	Cys
	290						295					300				
	Thr	Ser	Leu	Arg	Leu	Pro	Arg	Asp	His	Tyr	Val	Arg	Cys	Asp	Ser	Asn
	305					310					315					320
40																
	Asp	Tyr	Ser	Val	His	Pro	Gly	Val	Ile	Gly	His	Arg	Val	Leu	Val	Arg
					325					330					335	
45	Ala	Asp	Leu	Glu	Arg	Val	His	Val	Phe	Cys	Asp	Gly	Glu	Leu	Val	Ala
				340					345					350		
	Asp	His	Glu	Arg	Ile	Trp	Ala	Val	His	Gln	Thr	Val	Ser	Asp	Pro	Ala
			355					360					365			
50																
	His	Val	Glu	Ala	Ala	Lys	Val	Leu	Arg	Arg	Arg	His	Phe	Ser	Ala	Ala
	370						375					380				
55	Ser	Pro	Val	Val	Glu	Pro	Gln	Val	Gln	Val	Arg	Ser	Leu	Ser	Asp	Tyr
	385					390					395					400

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Asp Asp Ala Leu Gly Val Asp Ile Asp Gly Gly Val Ala
405 410

5

<210> 210

<211> 1239

10

<212> DNA

<213> Mycobacterium tuberculosis

15

<400> 210

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atcaagatga tcgcccgggt gctggggatt tccaagaaca cggatgaagtc agcggttgaa 120

20

tcaaaccagc agccgaaata tgaacgggca ccgcagggtt cgatcggtga tgcggttgag 180

ccgcggatcc gggagttggt gcaggcctat ccgacgatgc cggcgacggt gatcgccgag 240

cggatcggtt gggagcgctc gattcggttg ctctcggcgc ggggtggcga gctgcgcccg 300

25

gtgtatctgc cgccggaccc ggcgtcggc accacgtatg tggcaggcga aattgcccag 360

tgcgacttct ggtttccgcc gatcgagttg ccggtagggt tcgggcagac ccgcacggcc 420

aaacagttgc cgggtgctgac catggtgtgc gcctattcgc gctggctggt ggcgatgctg 480

30

ctgcccagca ggtgtgccga ggacctgttc gccggctggt ggcggctgat cgaggcggtg 540

ggggcggtgc cgcgggtggt ggtgtgggat ggcgagggcg cgatcgggcg ctggcgcggc 600

gggcggtcgg agttgaccac tgagtgtcag gcgttcgcgc gcacgctggc ggccaagggtg 660

35

ctcatctgcc ggccggccga ccggaggcc aagggcctca ttgaacgggc ccacgactac 720

ctggagcgct cgtttttgcc cgggcgggtg tttgcctcgc cggccgattt caacgcccac 780

ctgggcgcct ggctggcgct ggtgaacacc cgcaccgcgc gggcgctggg ttgtgcgccc 840

40

accgatcgca tcggcgcgga tcgggcccgc atgtgagct tgccgcccgt ggcgccggcc 900

accgggtggt gcacctcgct gcggctgccc cgggatcact atgtgcgctg cgattccaac 960

gactactcgg tgcaccggg tgtgatcggg catcggtgct tgggtgcgcgc cgacctggag 1020

45

cgggtgcatg tgttctgcga cgggtgagctg gtcgccgacc acgagcggtat ctgggcgggtc 1080

catcagacgg tctccgatcc cgcacatgtg gaggcgcgca aggtgttgcg ccgcccggac 1140

ttcagtgcag catcaccggt agttgagccg caggtgcagg tccgctcact gagcgactac 1200

50

gatgacgcgc tgggagtcga catcgatggc ggggtggcc 1239

<210> 211

<211> 153

55

<212> PRT

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<213> Mycobacterium tuberculosis

5 <400> 211

Val Asp Val Ile Trp Ser Ala Thr Ile Ala Thr Thr Val Ala Thr Gly
1 5 10 15

10 Met Arg Lys Pro Arg Met His Gly Met Pro Pro Ile Thr Ser Gly Ser
20 25 30

Met Val Thr Arg Val Thr Arg Met Ser Ile Arg Leu Ala Gly Asp Ser
35 40 45

15 Thr Leu Gly Arg Phe Ser Thr Ser Arg Leu Gly Leu Ser Ser Ala Lys
50 55 60

20 Ser Lys Pro Glu Gly Asp Phe Gly Thr Ala Cys Gly Ala Val Ser Gly
65 70 75 80

Gly Asp Ala Gly Val Val Ala Leu Ala Glu Gly Val Asp Asp Gly Gln
85 90 95

25 Ser Lys Pro Gly Ala Ala Gly Gly Ala Arg Gly Val Gly Gly Phe Arg
100 105 110

30 Glu Ser Arg Ala Asp Cys Gly Glu Gln Phe Gly Val Ala Ser Trp Thr
115 120 125

Pro Gln Gly Glu Phe Glu Phe Gly Gly Gln Glu Ala Lys Gly Val Arg
130 135 140

35 Ser Ser Trp Pro Ala Ser Leu Thr Asn
145 150

40 <210> 212

<211> 459

<212> DNA

45 <213> Mycobacterium tuberculosis

<400> 212

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tctataaggc tagccggtga cagcacgctg gggcggttct ccaccagccg tcttggttta 180

agtcagcca agagcaagcc ggagggagat ttcggcaccg cctgcggcgc ggtttcgggc 240

55 ggtgacgctg gcgtggttgc gttggctgag ggtgtcgacg atggccagtc caagcccggg 300

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gcccgcggtg gtgcgcgcgg tgtcggtggt ttccgcgaga gccgagcgga ttgcggcgag 360
 cagttcgggg ttgcttcgtg gacgccgcag ggcgagttcg agttcgggtg tcaggaggct 420
 5 aagggggtgc gaagttcgtg gcccgcacgc ctgacgaat 459

<210> 213

<211> 337

<212> PRT

<213> Mycobacterium tuberculosis

<400> 213

Val Ser Val Phe Ala Thr Ala Thr Gly Ile Gly Ser Trp Pro Gly Thr
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Leu Ala Tyr Leu Thr Glu Leu Pro Ala Arg Gly Val Gly Ala Asp Met
 35 40 45

Leu Gly Arg Ala Gly Gly Leu Leu Val Asp Val Ala Ile Asp Thr Val
 50 55 60

Pro Arg Gly Tyr Arg Ile Ala Ala Arg Pro Gly Ala Val Thr Arg Arg
 65 70 75 80

Ala Ala Ser Leu Leu Asp Glu Asp Met Asp Ala Leu Glu Glu Ala Trp
 85 90 95

Glu Thr Ala Gly Leu Arg Gly Cys Gly Arg Ala Val Lys Val Gln Ala
 100 105 110

Pro Gly Pro Val Thr Leu Val Ala Gly Leu Glu Leu Ala Asn Gly His
 115 120 125

Arg Ala Ile Thr Asp Pro Gly Ala Val Arg Asp Leu Ala Ala Ser Leu
 130 135 140

Ala Glu Gly Val Ala Ala His Arg Ala Ala Leu Ala Arg Arg Leu Asp
 145 150 155 160

Thr Pro Val Val Val Gln Phe Asp Glu Pro Ser Leu Pro Ala Ala Leu
 165 170 175

Gly Gly Arg Leu Thr Gly Val Thr Ala Leu Ser Pro Val Ala Pro Leu
 180 185 190

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Asp Glu Thr Val Ala Glu Ala Leu Leu Asp Thr Cys Ile Ala Ala Val
 195 200 205
 5 Asp Ala Asp Val Ala Leu His Ser Cys Ser Pro Asp Leu Pro Trp Asp
 210 215 220
 Leu Leu Gln Arg Ser Arg Ile Ser Ala Val Ser Val Asp Ala Ser Thr
 225 230 235 240
 10 Leu Gln Ala Ala Asp Leu Asp Ala Val Ala Ala Phe Val Glu Ser Gly
 245 250 255
 Arg Thr Val Val Leu Gly Leu Val Pro Val Thr Ala Pro Glu Arg Ala
 260 265 270
 15 Pro Ser Met Glu Glu Val Ala Ala Ala Val Ala Val Thr Asp Arg
 275 280 285
 20 Leu Gly Val Pro Arg Ser Ala Leu Arg Asp Arg Leu Gly Val Ser Pro
 290 295 300
 Ala Cys Gly Leu Ala Asn Ala Thr Gly Gln Trp Ala Arg Thr Ala Val
 305 310 315 320
 Gly Leu Ala Arg Asp Val Ala Glu Ala Phe Ala Arg Asp Pro Glu Ala
 325 330 335
 30 Ile
 35 <210> 214
 <211> 1011
 <212> PRT
 40 <213> Mycobacterium tuberculosis
 <400> 214
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 1 5 10 15
 Cys Gly Gly Cys Cys Ala Cys Cys Gly Gly Gly Ala Thr Cys Gly Gly
 20 25 30
 50 Ala Thr Cys Gly Thr Gly Gly Cys Cys Gly Gly Gly Cys Ala Cys Cys
 35 40 45
 55 Gly Cys Cys Gly Cys Gly Cys Gly Ala Gly Ala Gly Gly Cys Cys Gly
 50 55 60

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5 Cys Gly Cys Ala Gly Gly Thr Cys Gly Thr Gly Gly Thr Cys Gly Gly
65 70 75 80

Gly Gly Ala Gly Thr Thr Gly Gly Cys Gly Gly Gly Thr Gly Cys Ala
85 90 95

10 Thr Thr Gly Gly Cys Cys Thr Ala Thr Cys Thr Cys Ala Cys Cys Gly
100 105 110

15 Ala Gly Cys Thr Gly Cys Cys Cys Gly Cys Cys Ala Gly Gly Gly Gly
115 120 125

Cys Gly Thr Cys Gly Gly Cys Gly Cys Cys Gly Ala Cys Ala Thr Gly
130 135 140

20 Cys Thr Gly Gly Gly Gly Cys Gly Ala Gly Cys Cys Gly Gly Cys Gly
145 150 155 160

25 Gly Ala Cys Thr Gly Cys Thr Gly Gly Thr Cys Gly Ala Cys Gly Thr
165 170 175

Gly Gly Cys Gly Ala Thr Thr Gly Ala Cys Ala Cys Cys Gly Thr Gly
180 185 190

30 Cys Cys Thr Cys Gly Thr Gly Gly Cys Thr Ala Cys Cys Gly Cys Ala
195 200 205

35 Thr Cys Gly Cys Thr Gly Cys Thr Cys Gly Ala Cys Cys Cys Gly Gly
210 215 220

Cys Gly Cys Gly Gly Thr Gly Ala Cys Ala Cys Gly Gly Cys Gly Gly
225 230 235 240

40 Gly Cys Cys Gly Cys Gly Ala Gly Cys Cys Thr Cys Cys Thr Cys Gly
245 250 255

45 Ala Cys Gly Ala Gly Gly Ala Thr Ala Thr Gly Gly Ala Thr Gly Cys
260 265 270

Cys Thr Thr Ala Gly Ala Ala Gly Ala Gly Gly Cys Cys Thr Gly Gly
275 280 285

50 Gly Ala Gly Ala Cys Cys Gly Cys Gly Gly Gly Cys Cys Thr Gly Cys
290 295 300

55 Gly Thr Gly Gly Cys Thr Gly Thr Gly Gly Gly Cys Gly Gly Gly Cys
305 310 315 320

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	Gly	Gly	Thr	Gly	Ala	Ala	Gly	Gly	Thr	Gly	Cys	Ala	Gly	Gly	Cys	Gly	
					325					330					335		
5	Cys	Cys	Cys	Gly	Gly	Gly	Cys	Cys	Ala	Gly	Thr	Cys	Ala	Cys	Ala	Thr	
				340					345					350			
	Thr	Gly	Gly	Thr	Cys	Gly	Cys	Gly	Gly	Gly	Gly	Thr	Thr	Gly	Gly	Ala	
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	Gly	Cys	Thr	Gly	Gly	Cys	Cys	Ala	Ala	Cys	Gly	Gly	Thr	Cys	Ala	Cys	
		370					375					380					
15	Cys	Gly	Gly	Gly	Cys	Gly	Ala	Thr	Cys	Ala	Cys	Cys	Gly	Ala	Cys	Cys	
	385					390					395					400	
	Cys	Cys	Gly	Gly	Ala	Gly	Cys	Cys	Gly	Thr	Gly	Cys	Gly	Thr	Gly	Ala	
20					405					410					415		
	Cys	Cys	Thr	Gly	Gly	Cys	Cys	Gly	Cys	Cys	Thr	Cys	Gly	Cys	Thr	Gly	
				420					425					430			
25	Gly	Cys	Cys	Gly	Ala	Ala	Gly	Gly	Cys	Gly	Thr	Thr	Gly	Cys	Cys	Gly	
			435					440					445				
	Cys	Gly	Cys	Ala	Thr	Cys	Gly	Cys	Gly	Cys	Gly	Gly	Cys	Gly	Cys	Thr	
30		450				455						460					
	Gly	Gly	Cys	Gly	Cys	Gly	Thr	Cys	Gly	Ala	Cys	Thr	Thr	Gly	Ala	Cys	
	465					470					475					480	
	Ala	Cys	Ala	Cys	Cys	Gly	Gly	Thr	Gly	Gly	Thr	Gly	Gly	Thr	Gly	Cys	
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25 Gly Gly Thr Gly Gly Ala Thr Gly Cys Gly Ala Gly Cys Ala Cys Ala
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30 Cys Thr Gly Cys Ala Gly Gly Cys Thr Gly Cys Gly Gly Ala Thr Thr
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40 Cys Gly Ala Ala Cys Cys Gly Thr Cys Gly Thr Gly Cys Thr Gly Gly
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50 Cys Cys Thr Thr Cys Gly Ala Thr Gly Gly Ala Ala Gly Ala Gly Gly
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55 Cys Gly Cys Gly Gly Thr Cys Ala Cys Cys Gly Ala Thr Cys Gly Gly

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	Gly	Gly	Gly	Cys	Thr	Thr	Gly	Cys	Cys	Cys	Gly	Thr	Gly	Ala	Thr	Gly	
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35 <213> Mycobacterium tuberculosis

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50 Arg Asp Asp Val Ala Ala Val Ile Leu Tyr Gly Gly His Glu Ile Phe
50 55 60

Ser Ala Gly Asp Asp Met Pro Glu Leu Arg Thr Leu Ser Ala Gln Glu
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55 Ala Asp Thr Ala Ala Arg Ile Arg Gln Gln Ala Val Asp Ala Val Ala

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Glu Leu Gly Trp Leu Gly Leu His Ile Asp Glu Arg His Gly Gly Ser
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35 Gly Tyr Gly Leu Ser Glu Leu Val Val Val Ile Glu Glu Leu Gly Arg
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Gln Val Thr Asp Gly Val Ala Asp Gly Glu Ala Gly Ile Val Leu Gly
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165 170 175

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 30 Gln Val His Gly Gly Ile Gly Phe Thr Trp Glu His Asp Ala His Leu
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 35 His Leu Arg Arg Ala Leu Val Thr Val Gly Leu Phe Gly Gly Asp Ala
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 40 Ile Ser Leu Asp Leu Pro Ala Gln Ala Glu Glu Leu Arg Ala Arg Ile
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 405 410 415
 Phe Ser Ala Ala Gly Ile Glu Arg Pro Asp Tyr Ser Ile Thr Gly Trp
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 35 40 45
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	35 40 45		
	Ser Ala Ala Ile Ala Ala Leu Phe Ser Gly His Gly Gln Asp Tyr Gln		
55	50 55 60		

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His Asp Leu Ser Asp His Pro Pro Pro Asp Leu Thr Ala Leu Gly Leu
 35 35 40 45

Ala Ala Pro Arg Val Ser Pro Pro Ala Ser Leu Ala Ala Leu Cys Ser
 50 55 60

Ser Asp Leu Val Asp Arg Ala Gly His Ala Arg Gly Lys Ala Tyr Arg
 65 70 75 80

Asp Ile Ala Arg Asn Leu Gln Gly Gln Leu Asp His Leu Pro Asp Leu
 85 90 95

Ile Ala Arg Pro Arg Ser Glu Gln Asp Val Ile Asp Val Leu Asp Trp
 100 105 110

Cys Ala Arg Glu Gly Ile Ala Val Ile Pro Tyr Gly Gly Gly Ser Ser
 115 120 125

Val Val Gly Gly Val Glu Pro Arg Phe Asp Glu Pro Val Val Thr Val
 130 135 140

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	Asp	Val	Thr	Ala	Met	Ser	Ala	Val	Leu	Glu	Ile	Asp	Arg	Val	Ser	Arg	145	150	155	160
5	Ala	Ala	Arg	Ile	Gln	Ala	Gly	Ala	Phe	Gly	Pro	Ser	Ile	Glu	His	Gln	165	170	175	
	Leu	Arg	Pro	His	Asp	Leu	Thr	Leu	Arg	His	Phe	Pro	Gln	Ser	Phe	Gly	180	185	190	
10	Phe	Ser	Thr	Leu	Gly	Gly	Trp	Leu	Ala	Thr	Arg	Ser	Gly	Gly	His	Phe	195	200	205	
	Ala	Thr	Leu	Tyr	Thr	His	Ile	Asp	Asp	Leu	Thr	Glu	Ser	Leu	Arg	Ile	210	215	220	
	Val	Thr	Pro	Val	Gly	Ile	Ser	Glu	Ser	Arg	Arg	Leu	Pro	Gly	Ser	Gly	225	230	235	240
20	Ala	Gly	Pro	Ser	Pro	Asp	Arg	Leu	Phe	Leu	Gly	Ser	Glu	Gly	Thr	Leu	245	250	255	
	Gly	Ile	Ile	Thr	Glu	Ala	Trp	Met	Arg	Leu	Gln	His	Arg	Pro	Arg	Trp	260	265	270	
	Gln	Val	Thr	Val	Ser	Val	Val	Phe	Asp	Asp	Trp	Ala	Ala	Ala	Val	Ala	275	280	285	
30	Ala	Thr	Arg	Thr	Ile	Ala	Gln	Ala	Gly	Leu	Tyr	Pro	Ala	Asn	Cys	Arg	290	295	300	
	Leu	Leu	Asp	Pro	Ala	Glu	Ala	Leu	Leu	Asn	Ala	Gly	Thr	Ser	Val	Gly	305	310	315	320
35	Gly	Gly	Leu	Leu	Val	Leu	Ala	Phe	Glu	Ser	Ala	Asp	His	Pro	Ile	Asp	325	330	335	
	Pro	Trp	Leu	His	Arg	Ala	Val	Ala	Ile	Thr	Ala	Glu	His	Gly	Gly	Thr	340	345	350	
40	Val	Thr	Ala	Gln	Arg	Ser	Arg	Gly	Thr	Thr	Ser	Asp	Ala	Thr	Glu	His	355	360	365	
	Asn	Ala	Ala	Ala	Asn	Trp	Arg	Ser	Ala	Phe	Leu	Arg	Met	Pro	Tyr	Gln	370	375	380	
50	Arg	Asp	Ala	Leu	Val	Arg	Arg	Gly	Val	Ile	Ala	Glu	Thr	Phe	Glu	Thr	385	390	395	400
	Ala	Cys	Thr	Trp	Asp	Gly	Phe	Asp	Thr	Leu	His	Ala	Ala	Val	Thr	Asp	405	410	415	
55																				

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Ala Ala Arg Thr Ala Ile Trp Lys Val Cys Gly Thr Gly Val Val Thr
420 425 430

5

Cys Arg Phe Thr His Val Tyr Pro Asp Gly Pro Ala Pro Tyr Tyr Gly
435 440 445

10

Ile Tyr Ala Gly Gly Arg Trp Gly Ser Leu Asp Ala Gln Trp Asp Glu
450 455 460

Ile Lys Ala Ala Val Ser Glu Ala Ile Ser Ala Ser Gly Gly Thr Ile
465 470 475 480

15

Thr His His His Ala Val Gly Arg Asp His Arg Ala Trp Tyr Asp Arg
485 490 495

20

Gln Arg Pro Asp Pro Phe Ala Ala Ala Leu Arg Ala Ala Lys Ser Ala
500 505 510

Leu Asp Pro Ala Gly Ile Leu Asn Pro Gly Val Leu Leu Gly Arg
515 520 525

25

<210> 226
<211> 1581
<212> DNA

30

<213> Mycobacterium tuberculosis

35

<400> 226
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ccggacctga ccgcgctcgg tttggcggcc ccacgggtca gcccgccggc atcgttgccc 180
gcgctctgct caagcgatct cgtcgatcgg gccggacacg cgcgcggcaa agcgtatcgc 240
gacatcgcac gcaacctgca gggccagctc gaccacctgc ccgacctcat cgcgccaccc 300
cgcagcgagc aggacgtgat cgacgtgctg gattggtgtg cgcgcgaggg gattgcggtc 360
atcccatacg gtggtggcag ctcggtggtt ggcggtgtcg agccgcgctt cgatgagccg 420
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gccgcgcgca tccaggcggg tgcgttcggc ccctcgatcg agcatcagct tcgcccacac 540
gatttgacac tgcgccattt cccgcagtc ttcggcttct cgactctcgg tggctggttg 600
gccaccgct cggcgggaca cttegccacg ctctataccc atatcgacga cttgaccgaa 660
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gccggaccat ccccgaccg gttgttctc ggggtccgagg ggacgcttg catcatcacc 780
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gaggcgtgga tgcggctgca acaccgtccg cgatggcagg tcacggtgtc cgtggtgttt 840

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5 gacgactggg ccgccgcggt cgcgcgcgacc cggacgacg ctcaggcggg gctgtaccgc 900
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 10 actacaagcg acgcaacgga acacaacgca gccgcgaact ggcgctcggc gtttctgcgc 1140
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 gcttgacact gggacggatt cgatactcta catgccgcgg tgaccgatgc cgctcggacc 1260
 15 gcgatctgga aggtatgcgg gaccggagta gtgacctgtc gattcaccca tgtctaccgc 1320
 gacggcccg ctccttacta cggcatctat gccggcgggc gctgggggtc gctcgacgcg 1380
 cagtgggacg agatcaagc tgccgtgtcc gagcgatca gcgccagtgg cggtaaccac 1440
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 ccgttcgcgg cggccctgcg ggcggcgaag tccgcactcg acccgcccg gatcctcaac 1560
 ccagggtgt tgctcggtcg c 1581

 25 <210> 227
 <211> 145
 <212> PRT
 30 <213> Mycobacterium tuberculosis

 <400> 227
 35 Met Thr Ser Phe Ala His Pro Gly Thr Arg Gly Leu Ser Thr Val Phe
 1 5 10 15
 Gly Leu Met Met Val Gly Ser Ala Ala Val Gly Ser His Gly Leu Ala
 20 25 30
 40 Val Val Val Gly Leu Ala Ala Val Ile Ala Val Gly Val Ala Ala Val
 35 40 45
 45 Phe Arg Leu Ala Ala Thr Leu Ala Val Val Leu Ser Val Val Met Ile
 50 55 60
 Val Val Ser Gly Pro Thr His Val Leu Ala Ala Leu Ser Gly Phe Cys
 65 70 75 80
 50 Ala Ala Val Tyr Leu Val Cys Arg Tyr Gly Ala Gly Val Val Ala Gly
 85 90 95
 55 Ser Trp Pro Thr Thr Val Ala Ala Val Gly Phe Thr Phe Ala Gly Leu
 100 105 110

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Ala Ala Thr Ser Phe Pro Leu Gln Val Pro Trp Leu Pro Leu Ala Ala
115 120 125

5 Pro Leu Ala Val Leu Ala Thr Tyr Val Leu Ala Thr Arg Pro Phe Ser
130 135 140

10 Arg
145

<210> 228
<211> 435

15 <212> DNA
<213> Mycobacterium tuberculosis

20 <400> 228
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gtgggggtcgg ccgctgtggg atcgcacggg ctggctgttg tcgtggggct tgccgcgggtg 120
attgcggtag ggggtggcggc ggtgtttcgc ctggcgcaa cgcttgccgt ggtgtttgtcg 180
25 gtggtgatga tcgtggtgtc cgccccgacg catgtgcttg ccgcattgtc ggggttttgc 240
gccgccgtct acctggtgtg ccgatacggg gccggtgttg tcgccgggag ctggccgacg 300
accgttgccg ccgttggttt cacgttcgct ggggtggctg cgacgtcgtt cccgctgcaa 360
30 gtgccatggc tgccgttggc ggcaccgttg gccgtgttgg ctacctacgt gctggccacc 420
cgtccgttct cgagg 435

35 <210> 229
<211> 119
<212> PRT
40 <213> Mycobacterium tuberculosis

<400> 229

45 Leu Arg Leu Gly Ala Gly Phe Arg Lys Pro Val Pro Thr Leu Leu Leu
1 5 10 15

Glu His Arg Ser Arg Lys Ser Gly Lys Asn Phe Val Ala Pro Leu Leu
20 25 30

50 Tyr Ile Thr Asp Arg Asn Asn Val Ile Val Val Ala Ser Ala Leu Gly
35 40 45

55 Gln Ala Glu Asn Pro Gln Trp Tyr Arg Asn Leu Pro Pro Asn Pro Asp
50 55 60

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5 Thr His Ile Gln Ile Gly Ser Asp Arg Arg Pro Val Arg Ala Val Val
 65 70 75 80
 Ala Ser Ser Asp Glu Arg Ala Arg Leu Trp Pro Arg Pro Val Asp Ala
 85 90 95
 10 Tyr Ala Asp Phe Asp Ser Cys Gln Ser Trp Thr Glu Arg Gly Ile Pro
 100 105 110
 Val Ile Ile Leu Arg Pro Arg
 115
 15
 <210> 230
 <211> 357
 20 <212> DNA
 <213> Mycobacterium tuberculosis
 25 <400> 230
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 cgcaagtcocg gcaagaactt cgtcgcacca ctgctttaca tcaccgaccg taacaatgtc 120
 atcgtcgttg cctctgccct tgggcaggca gaaaaccgc agtggtatcg caacctgccg 180
 30 cccaatcccg acaccacat tcagatcgga tccgatcgcc gcccggtgag agccgtcgtg 240
 gccagctcgg acgagcgggc gcgcctatgg ccgcgccag tagacgccta cgccgacttc 300
 gattcttgcc aaagctggac cgagcgtggg attccggtga tcattcttgcg gccacgc 357
 35
 <210> 231
 <211> 108
 40 <212> PRT
 <213> Mycobacterium tuberculosis
 45 <400> 231
 Met Ser Gly Gly Ser Ser Arg Arg Tyr Pro Pro Glu Leu Arg Glu Arg
 1 5 10 15
 50 Ala Val Arg Met Val Ala Glu Ile Arg Gly Gln His Asp Ser Glu Trp
 20 25 30
 Ala Ala Ile Ser Glu Val Ala Arg Leu Leu Gly Val Gly Cys Ala Glu
 35 40 45
 55 Thr Val Arg Lys Trp Val Arg Gln Ala Gln Val Asp Ala Gly Ala Arg

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	50	55	60	
5	Pro Gly Thr Thr Thr	Glu Glu Ser Ala Glu	Leu Lys Arg Leu Arg Arg	
	65	70	75	80
	Asp Asn Ala Glu Leu Arg Arg Ala Asn Ala	Ile Leu Lys Thr Ala Ser		
		85	90	95
10	Ala Phe Phe Ala Ala Glu Leu Asp Arg Pro Ala Arg			
		100	105	
15	<210> 232			
	<211> 324			
	<212> DNA			
20	<213> Mycobacterium tuberculosis			
	<400> 232			
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25	gtcgcagaga tccgcggtca gcacgattcg gagtgggcag cgatcagtga ggtcgcccgt		120	
	ctacttggtg ttggctgcgc ggagacggtg cgtaagtggg tgcgccaggc gcaggtcgat		180	
	gccggcgcac ggcccgggac caccgaccgaa gaatccgctg agctgaagcg cttgcggcgg		240	
30	gacaacgcgc aattgcgaag ggogaacgcg attttaaaga ccgcgtcggc tttcttcgcg		300	
	gccgagctcg accggccagc acgc		324	
35	<210> 233			
	<211> 133			
	<212> PRT			
40	<213> Mycobacterium tuberculosis			
	<400> 233			
45	Met Pro Asp Val Asp Trp Asn Met Leu Arg Gly Asn Ala Thr Gln Ala			
	1	5	10	15
	Ala Ala Gly Ala Tyr Val Pro Tyr Ser Arg Phe Ala Val Gly Ala Ala			
		20	25	30
50	Ala Leu Val Asp Asp Gly Arg Val Val Thr Gly Cys Asn Val Glu Asn			
		35	40	45
55	Val Ser Tyr Gly Leu Thr Leu Cys Ala Glu Cys Ala Val Val Cys Ala			
		50	55	60

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Leu His Ser Thr Gly Gly Gly Arg Leu Leu Ala Leu Ala Cys Val Asp
 65 70 75 80

5

Gly His Gly Ser Val Leu Met Pro Cys Gly Arg Cys Arg Gln Val Leu
 85 90 95

10

Leu Glu His Gly Gly Ser Glu Leu Leu Ile Asp His Pro Val Arg Pro
 100 105 110

Arg Arg Leu Gly Asp Leu Leu Pro Asp Ala Phe Gly Leu Asp Asp Leu
 115 120 125

15

Pro Arg Glu Arg Arg
 130

20

<210> 234
 <211> 399
 <212> DNA
 <213> Mycobacterium tuberculosis

25

<400> 234
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 tatgtgccgt attcgcgggt tgcgggtgggt gcggccgcac tggtcgacga tggtcgcgtg 120
 gtgaccggat gcaacgtgga aaacgtctcg tatggcttga ctttgtgcgc cgaatgtgcg 180
 gtggtgtgcg ccttgcattc gaccggcggc ggccggctgc tcgcgtggc ctgcgtcgac 240
 ggccatggat ccgtgctgat gccgtgcggg cgatgccgtc aggtgctgct cgaacacggg 300
 gggtccgagc tactgatcga ccatccggtg cgaccccgcc ggctcggcga cctgctgccc 360
 gacgccttcg gcctcgacga cctcccccg gaacgccgg 399

30

<210> 235
 <211> 204
 <212> PRT

35

<213> Mycobacterium tuberculosis

40

<400> 235

45

Met Ser Val Gln Thr Asp Pro Ala Leu Arg Glu His Pro Asn Arg Val
 1 5 10 15

50

Asp Trp Asn Ala Arg Tyr Glu Arg Ala Gly Ser Ala His Ala Pro Phe
 20 25 30

55

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Ala Pro Val Pro Trp Leu Ala Asp Val Leu Arg Ala Gly Val Pro Asp
35 40 45

5 Gly Pro Val Leu Glu Leu Ala Ser Gly Arg Ser Gly Thr Ala Leu Ala
50 55 60

Leu Ala Ala His Gly Arg Gln Val Thr Ala Ile Asp Val Ser Asp Val
65 70 75 80

10 Ala Leu Leu Gln Leu Asp Ser Glu Ala Val Arg Arg Gly Val Ala Asp
85 90 95

15 Arg Leu Asn Leu Val Gln Ala Asp Leu Gly Cys Trp Glu Pro Gly Glu
100 105 110

Thr Arg Phe Ala Leu Val Leu Ser Arg Leu Phe Trp Asp Ala Ala Ile
115 120 125

20 Phe His Arg Ala Cys Glu Ala Val Met Pro Gly Gly Val Leu Ala Trp
130 135 140

Glu Ser Leu Ala Leu Ser Gly Ala Glu Ala Gly Thr Ala Ser Ala Lys
145 150 155 160

25 Arg Arg Val Lys Pro Gly Glu Pro Ala Cys Leu Leu Pro Ala Asp Phe
165 170 175

30 Thr Val Val His Glu Gly Gln Gly Asn Cys Asp Ser Ala Pro Ser Arg
180 185 190

Ile Met Ile Ala Arg Arg Ser Pro Leu Pro Gly Ala
195 200

35 <210> 236

40 <211> 612

<212> DNA

<213> Mycobacterium tuberculosis

45 <400> 236

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cgatacgaac gcgcgggttc ggcgcacgcg ccgtttgccc cgggtgccttg gctcgcgat 120

50 gtcctcagag caggcgttcc ggacgggtccc gttctggagt tagccagcgg tcgatcgggt 180

accgcactgg cgttggccgc ccacggccgc caggtcaccg caatcgatgt gtccgatgtc 240

gcgctgctgc agctggacag cgaggccgtg cgtcgaggcg tggccgatcg gctcaacctc 300

55 gtgcaggccg acttgggctg ctgggaaccc ggcgagacgc gtttcgcgct ggtgctcagc 360

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aggctctttt gggatgcggc gatatttcac cgcgcctgtg aggcgggtgat gccaggcggc 420
 gtattggcat gggagtcgct ggctctcagt ggcccgagg cgggcacagc cagcgcaag 480
 5 cgacgtgtca agccgggaga gccagcgtgt ctgcttcctg ccgacttcac cgttgtacac 540
 gaggggcagg gtaactgcga ttcgggcgcg tcgcggatca tgatcgcgcg gcgctcaccg 600
 10 ttgccagggg ca 612

 <210> 237
 <211> 264
 15 <212> PRT
 <213> Mycobacterium tuberculosis

 20 <400> 237
 Val Leu Ala Ser Cys Pro Ala Arg Ser Gly Ala Ala Val Ala Asp Ala
 1 5 10 15
 25 Ile Lys Ser Ala Val Gly Val Gln Pro Ser Gly Val Glu His Lys Thr
 20 25 30
 Leu Arg Arg Met Asp Leu Val Arg Tyr Leu Ala Gly Gly His Thr Thr
 30 35 40 45
 Tyr Pro Pro Glu Gly Phe Val Ala Gly Ser Asp Val Ile Gly Thr Thr
 50 55 60
 35 Asn Pro Ala Ala Ala Gln Ala Ile Val Ala Ala Ile Gly Thr Trp Pro
 65 70 75 80
 Pro Ala Ala Gly Arg Ala Ser Ala Leu Ile Asp Ser Leu Gly Gly Ala
 40 85 90 95
 Val Gly Asp Met Asp Pro Glu Gly Ser Ala Phe Pro Trp Cys Arg Gln
 100 105 110
 45 Ser Ala Val Val Gln Trp Tyr Val Asn Thr Pro Ser Asp Gly Gln Val
 115 120 125
 Ala Thr Ala Asn Lys Trp Leu Ser Asp Ala His His Ala Val Gln His
 50 130 135 140
 Phe Ser Val Gly Gly Tyr Val Asn Tyr Leu Glu Ala Asn Ala Ala Ala
 145 150 155 160
 55 Ser Gln Tyr Phe Gly Ala Asn Leu Ser Arg Leu Thr Thr Val Arg Arg
 165 170 175

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5 Lys Tyr Asp Pro Asp Arg Ile Met Tyr Ser Gly Leu Asp Phe Ser Thr
 180 185 190
 Arg Gln Val Ala Glu Arg Leu Leu Pro Ala Leu Gly Phe Arg Val Arg
 195 200 205
 10 Phe Gly Val Leu Val Ile Arg Cys Ala Leu Cys Thr Asp Thr Val Lys
 210 215 220
 Arg Leu Gly Thr Leu Pro Asn Leu Thr Trp Ser Arg Leu Lys Val Asn
 15 225 230 235 240
 Val Ala Val Thr Gln Glu Gln Ala Gly Val Met Asp Leu Pro Ala Leu
 245 250 255
 20 Pro Val Arg Arg Thr Pro Arg Arg
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 <210> 238
 <211> 792
 25 <212> DNA
 <213> Mycobacterium tuberculosis
 30 <400> 238
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 gttggagtgc aaccagtg agttgagcac aagacgctgc gccgtatgga cctggtgagg 120
 35 tatctggccg gcggccatac gacctatccg ccggagggct tcgtggctgg atccgatgtc 180
 atcgggaaga cgaatccggc cgcggcccaa gccatcgctc ccgccatcgg aacatggcca 240
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 40 gacccggagg gctcggcatt tccctgggtc cgccagtcgc ctgtggtgca gtggtatgtc 360
 aacaccccca gcgacggcca ggtggcgacg gccacaatat ggctgagcga cgcacaccac 420
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 gaccggatca tgtactcggg tctggatttc tctaccagac aggtcgtctga acgactttta 600
 cccgctctcg gctttcgagt gaggttcggg gttttggtta tcagggtgcgc actgtgcact 660
 50 gacactgtga aacgcttggt aactttgcc aaccttacgt ggtcgcgctt aaaagtgaac 720
 gtcgcagtga cccaagaaca ggctggggtc atggatttgc cggcgctgcc ggttcggcgc 780
 acgcccgggc gg 792
 55 <210> 239

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<211> 123

<212> PRT

<213> Mycobacterium tuberculosis

<400> 239

Val Ser Ala Ala Thr Asp Leu Tyr Ala Val His Gln Ala Leu Ala Gly
1 5 10 15

Glu Ser Arg Ala Ile Pro Thr Gly Ser Cys Pro Thr Val Gly Val Ala
20 25 30

Gly Leu Thr Leu Gly Gly Gly Leu Gly Ala Asp Ser Arg His Ala Gly
35 40 45

Leu Thr Cys Asp Ala Leu Lys Ser Ala Thr Val Val Leu Pro Gly Gly
50 55 60

Asp Ala Val Ser Ala Ser Ala Asp Asp His Ala Glu Leu Phe Trp Ala
65 70 75 80

Leu Arg Gly Gly Gly Gly Gly Asn Phe Gly Val Thr Thr Ser Met Thr
85 90 95

Phe Ala Arg Phe Pro Thr Ala Asp Cys Asp Val Val Arg Val Asp Phe
100 105 110

Ala Pro Ser Ala Ala Ala Gln Val Leu Val Gly
115 120

<210> 240

<211> 369

<212> DNA

<213> Mycobacterium tuberculosis

<400> 240

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ggcgccgatt ctgccatgc ggggttgacc tgcgatgcgc tcaagtcggc gacggtggtg 180

ttgcccggcg gtgatgcgt gagegcgtct gccgacgacc acgcggagct gttctgggcg 240

cttcgtggcg gcgggggcg caacttcggg gtgacgacat cgatgacgtt cgcgaggttc 300

cccaccgcg actgcgatgt cgtccgtgtc gatttcgcgc cgtctgcggc cgcgcaggtg 360

ctggtcggc

369

5 <210> 241
 <211> 213
 <212> PRT
 <213> Mycobacterium tuberculosis
 10
 <400> 241
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 Gly Gly Asp Ile Glu Ala Glu Met Cys Gln Leu Met Arg Glu Gln Pro
 20 25 30
 Pro Ala Glu Gly Val Val Asp Arg Val Ala Leu Gln Arg His Arg Asn
 35 40 45
 Val Ala Leu Ile Thr Leu Ser His Pro Gln Ala Gln Asn Ala Leu Asn
 50 55 60
 Leu Ala Ser Trp Arg Arg Leu Lys Arg Leu Leu Asp Asp Leu Ala Gly
 65 70 75 80
 30 Glu Ser Gly Leu Arg Ala Val Val Leu Arg Gly Ala Gly Asp Lys Ala
 85 90 95
 Phe Ala Ala Gly Ala Asp Ile Lys Glu Phe Pro Asn Thr Arg Met Ser
 100 105 110
 35 Ala Ala Asp Ala Ala Glu Tyr Asn Glu Ser Leu Ala Val Cys Leu Arg
 115 120 125
 40 Ala Leu Thr Thr Met Pro Ile Pro Val Ile Ala Ala Val Arg Gly Leu
 130 135 140
 Ala Val Gly Gly Gly Cys Glu Leu Ala Thr Ala Cys Asp Val Cys Ile
 145 150 155 160
 45 Ala Thr Asp Asp Ala Arg Phe Gly Ile Pro Leu Gly Lys Leu Gly Val
 165 170 175
 50 Thr Thr Gly Phe Thr Glu Ala Asp Thr Val Ala Arg Leu Ile Gly Pro
 180 185 190
 Ala Ala Leu Lys Tyr Leu Leu Phe Ser Gly Glu Leu Ile Gly Ile Glu
 195 200 205
 55

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Glu Ala Ala Arg Trp
210

5 <210> 242
<211> 639
<212> DNA
10 <213> Mycobacterium tuberculosis

<400> 242
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gtcgcgctgc aacgccatcg aaacgttgcg ttgatcacgc tgagccatcc gcaggcgcag 180
20 aacgcactca acctggcgag ctggcgctcg ctgaagcggc tgctggacga tctcgccggc 240
gaatcggggc tgcggggcgt ggtgctgcgg ggcgccggtg acaaggcgtt cgcgcggggt 300
gccgacatca aggagtttcc gaacaccgcg atgagcgcgg cggacgccgc ggagtacaac 360
25 gagagcctgg ccgtctgcct gagggcggtt accacgatgc cgatcccagt catcgcggcg 420
gtccgggggc tcgccgtcgg tggcggtgtg gagctggcga cggcctgcga tgtgtgcatc 480
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agcggagaac tgatcggcct tgaggaagcc gcccgtctgg 639

<210> 243
35 <211> 467
<212> PRT
<213> Mycobacterium tuberculosis

40 <400> 243

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Ala Ala Glu His Thr Gln Ser Tyr Val Arg Ala Cys His Leu Val Gly
20 25 30
50 Tyr Gln His Pro Asp Leu Thr Ala Tyr Pro Ala Gln Ile His Asp Trp
35 40 45
Tyr Gly Ser Glu Asp Gly Leu Asp Leu His Ala Leu Asp Ala Asp Cys
55 50 55 60

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	Ala	Gln	Leu	Arg	Ala	Ala	Ala	Ser	Val	Leu	Met	Glu	Ala	Leu	Arg	Met	
	65					70					75					80	
5	Glu	Arg	Ser	Gln	Val	Ala	Val	Leu	Ala	Ala	Ala	Trp	Thr	Gly	Ser	Gly	
					85					90					95		
	Ala	Asp	Ala	Ala	Val	His	Phe	Val	Gln	Arg	His	Cys	Glu	Thr	Gly	Asn	
10				100					105					110			
	Ser	Val	Val	Thr	Glu	Val	Arg	Ala	Ala	Ala	Gln	Arg	Cys	Glu	Ser	Leu	
			115					120					125				
15	Arg	Asp	Asn	Leu	Trp	Gln	Leu	Val	Asp	Ser	Lys	Val	Ala	Thr	Ala	Ile	
		130					135					140					
	Ala	Ile	Asp	Glu	Arg	Ala	Leu	Ala	Gln	Arg	Pro	Ala	Trp	Leu	Ala	Ala	
20	145					150					155					160	
	Ala	Glu	Ala	Leu	Thr	Thr	Glu	Gly	Ala	Asp	Arg	Pro	Thr	Ala	Val	Glu	
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		290					295					300					
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55	Ala	Val	Asp	Ser	Leu	Leu	Gly	Ser	Val	Ala	Glu	Gln	Leu	Gly	Asp	Pro	
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 Val Ala Glu Glu Ala Thr Leu Pro Pro Val Ala Glu Pro Pro Pro Pro
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<211> 404

30 <212> PRT

<213> Mycobacterium tuberculosis

35 <400> 245

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Pro Val Ala Val Asp Ser Leu Trp Phe Asp Ala Leu Arg Ser Val Ala
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<213> Mycobacterium tuberculosis

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 30 Gly Tyr Leu Ser Val Leu Arg Thr Ser Val Thr Gln Thr Ala Ala Ala
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 35 Gln His Val Ser Leu Asn Trp Thr His Pro Asp Pro Ala Thr Leu Trp
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 Thr Leu Ile Gly Gly Pro Arg Met Trp Glu Arg Arg Pro Gly Ala Ala
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 40 Asp Phe Cys Arg Ile Arg Val Gly Val Gly Ser Ala Pro Leu Ala Thr
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 45 Arg Leu Val Val Gly Gln Leu Pro Pro Ala Gln Arg Ala Asp Pro Val
 165 170 175
 Thr Arg Ala Ala Leu Arg Cys Phe Leu Ala Ala His Ala Thr Ile Ala
 180 185 190
 50 Asp Ala Pro Ile Ala Ile Pro Leu Arg Val Gly Gly Pro Ile Ala Ile
 195 200 205
 55 Asp Gly Asp Pro Thr Lys Val Arg Gly Leu Leu Arg Ala Met Ile Cys
 210 215 220

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5 Gln Leu Ala Val Trp His Ser Pro Glu Glu Leu Leu Ile Ala Gly Val
 225 230 235 240
 Val Ser Asp Arg Asn Arg Ala His Trp Asp Trp Leu Lys Trp Leu Pro
 245 250 255
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 260 265 270
 15 Val Tyr Ser Thr Leu Ala Glu Met Gln Asn Ala Leu Ala Ala Thr Val
 275 280 285
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 290 295 300
 20 Ala Ile Thr Gly Val Ile Thr Ile Glu Val Gly Ala Arg Arg Asp Gly
 305 310 315 320
 25 Ala Pro Pro Val Val Arg Cys Ala Gly Glu Val Thr Ala Leu Ala Cys
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 370 375 380
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 435 440 445
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	Ala	Ile	Gly	Arg	Val	Gly	Arg	Ser	Leu	Gly	Met	His	Leu	Leu	Leu	Ala
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 Ser Val Ala Arg Tyr Arg Gln Leu Arg Ala Lys Ser Ala Ala Glu Ser
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 Phe Ala Asp Ile Phe Leu Val Ile Asp Gly Trp Ala Ser Leu Arg Gln
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	Tyr	Val	Ser	Ser	Pro	Ala	Ala	Leu	Gly	Ala	Lys	Leu	Ser	Ser	Leu
	1085						1090					1095			
20	Val	Asp	Leu	Leu	Gln	Ala	Arg	Met	Pro	Ala	Pro	Asp	Val	Ser	Gln
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	Ala	Gln	Leu	Arg	Ala	Arg	Ser	Trp	Trp	Ser	Gly	Pro	Asp	Ile	Tyr
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 <213> Mycobacterium tuberculosis

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Glu Ser Ala Trp Ala Ile Cys Asp Ser Asp Asn Gly Gly Ser Thr Thr
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<212> DNA

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<213> Mycobacterium tuberculosis

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Leu Thr Thr Phe Thr Met Asp Thr Asn Thr Glu Ile Ala Val Ala Arg
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gcctacatcc atggcatgaa cggcatcgcg gagggggtgc ggcagctcgc cggcacctcg     1080
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<210> 257

<211> 391

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<212> PRT

<213> Mycobacterium tuberculosis

<400> 257

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      20             25             30
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10	Glu	Gln	Ser	Asn	Asn	Ile	Ser	Arg	Val	Ala	Trp	Leu	Thr	Ala	Gly	Leu	
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5 Lys Ile Gly Asp Ile Asp Ile Val Glu Ile Asn Glu Ala Phe Ala Ser
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 325 330 335
 10 Asn Val Asn Gly Gly Ala Ile Ala Leu Gly His Pro Val Gly Cys Thr
 340 345 350
 Gly Ser Arg Leu Ile Thr Thr Ala Leu His Glu Leu Glu Arg Thr Asp
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35 40 45
30 Val Ile Leu Arg Ala Glu Gly Arg Gly Phe Asn Ala Gly Val Asp Ile
50 55 60
35 Lys Glu Met Gln Arg Thr Glu Gly Phe Thr Ala Leu Ile Asp Ala Asn
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Arg Gly Cys Phe Ala Ala Phe Arg Ala Val Tyr Glu Cys Ala Val Pro
85 90 95
40 Val Ile Ala Ala Val Asn Gly Phe Cys Val Gly Gly Gly Ile Gly Leu
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45 Val Gly Asn Ser Asp Val Ile Val Ala Ser Glu Asp Ala Thr Phe Gly
115 120 125
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130 135 140
50 Arg Leu Val Pro Gln His Leu Met Arg Arg Leu Phe Phe Thr Ala Ala
145 150 155 160
55 Thr Val Asp Ala Ala Thr Leu Gln His Phe Gly Ser Val His Glu Val
165 170 175

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Val Ser Arg Asp Gln Leu Asp Glu Ala Ala Leu Arg Val Ala Arg Asp
180 185 190

5

Ile Ala Ala Lys Asp Thr Arg Val Ile Arg Ala Ala Lys Glu Ala Leu
195 200 205

10

Asn Phe Ile Asp Val Gln Arg Val Asn Ala Ser Tyr Arg Met Glu Gln
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Asp Ala Phe Val Lys Lys Ser
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<213> Mycobacterium tuberculosis

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gtgaacggat tctgcgtggg cggcgccatc ggctggctcg gcaactccga cgtcatcgctg 360
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<212> PRT
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15	Thr Asp Gly Glu Ala Gln Leu Leu Ala Asp Thr Pro Ala Leu Gly Lys	50 55 60
20	Thr Gly Ala Pro Asn Arg Ile Glu Gly Trp Met Pro Phe Gly Arg Val	65 70 75 80
	Phe Glu Thr Leu Ala Trp Gly Arg Arg His Val Val Met Gly Ala Asn	85 90 95
25	Gln Val Asp Arg Tyr Gly Asn Gln Asn Ile Ser Ala Phe Gly Pro Leu	100 105 110
30	Gln Arg Pro Thr Arg Gln Met Phe Gly Val Arg Gly Ser Pro Gly Asn	115 120 125
	Thr Ile Asn His Ala Thr Ser Tyr Trp Val Gly Asn His Cys Lys Arg	130 135 140
35	Val Phe Val Glu Ala Val Asp Val Val Ser Gly Ile Gly Tyr Asp Lys	145 150 155 160
40	Val Asp Pro Asp Asn Pro Ala Phe Arg Phe Val Asn Val Tyr Arg Val	165 170 175
	Val Ser Asn Leu Gly Val Phe Asp Phe Gly Gly Pro Asp His Ser Met	180 185 190
45	Arg Ala Val Ser Leu His Pro Gly Val Thr Pro Gly Asp Val Arg Asp	195 200 205
50	Ala Thr Ser Phe Glu Val His Asp Leu Asp Ala Ala Glu Gln Thr Arg	210 215 220
	Leu Pro Thr Asp Asp Glu Leu His Leu Ile Arg Ala Val Ile Asp Pro	225 230 235 240
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 tatggcaatc agaacatctc ggcgttcggg ccgtgcagc ggcgcaccgc gcagatgttc 360
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 50 Leu Val Asn Leu Ser Ala Gly Gln Pro Ser Ala Gly Ala Pro Glu Pro
 35 40 45
 55 Val Arg Ala Ala Ala Ala Ala Ala Leu His Leu Asn Gln Leu Gly Tyr
 50 55 60

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Ser Val Ala Leu Gly Ile Pro Glu Leu Arg Asp Ala Ile Ala Ala Asp
 65 70 75 80
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 Tyr Gln Arg Arg His Gly Ile Thr Val Glu Pro Asp Ala Val Val Ile
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 Thr Thr Gly Ser Ser Gly Gly Phe Leu Leu Ala Phe Leu Ala Cys Phe
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 Asp Ala Gly Asp Arg Val Ala Met Ala Ser Pro Gly Tyr Pro Cys Tyr
 115 120 125
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 Arg Asn Ile Leu Ser Ala Leu Gly Cys Glu Val Val Glu Ile Pro Cys
 130 135 140
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 Gly Pro Gln Thr Arg Phe Gln Pro Thr Ala Gln Met Leu Ala Glu Ile
 145 150 155 160
 Asp Pro Pro Leu Arg Gly Val Val Val Ala Ser Pro Ala Asn Pro Thr
 165 170 175
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 Gly Thr Val Ile Pro Pro Glu Glu Leu Ala Ala Ile Ala Ser Trp Cys
 180 185 190
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 Asp Ala Ser Asp Val Arg Leu Ile Ser Asp Glu Val Tyr His Gly Leu
 195 200 205
 Val Tyr Gln Gly Ala Pro Gln Thr Ser Cys Ala Trp Gln Thr Ser Arg
 210 215 220
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 Asn Ala Val Val Val Asn Ser Phe Ser Lys Tyr Tyr Ala Met Thr Gly
 225 230 235 240
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 Trp Arg Leu Gly Trp Leu Leu Val Pro Thr Val Leu Arg Arg Ala Val
 245 250 255
 Asp Cys Leu Thr Gly Asn Phe Thr Ile Cys Pro Pro Val Leu Ser Gln
 260 265 270
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 Ile Ala Ala Val Ser Ala Phe Thr Pro Glu Ala Thr Ala Glu Ala Asp
 275 280 285
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 Gly Asn Leu Ala Ser Tyr Ala Ile Asn Arg Ser Leu Leu Leu Asp Gly
 290 295 300
 Leu Arg Arg Ile Gly Ile Asp Arg Leu Ala Pro Thr Asp Gly Ala Phe
 305 310 315 320
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 Tyr Val Tyr Ala Asp Val Ser Asp Phe Thr Ser Asp Ser Leu Ala Phe

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	Asp Phe Asp Thr Ala Arg Gly Gly Ser Phe Val Arg Ile Ser Phe Ala	355		360		365	
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<210> 265

<211> 291

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<212> PRT

<213> Mycobacterium tuberculosis

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<400> 265

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Val Gly Asn Asp Gln Thr Val Val Leu Leu His Gly Gly Gly Pro Gly
35 40 45

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Ala Ala Ser Trp Thr Asn Phe Ser Arg Asn Ile Ala Val Leu Ala Arg
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His Phe His Val Leu Ala Val Asp Gln Pro Gly Tyr Gly His Ser Asp
65 70 75 80

Lys Arg Ala Glu His Gly Gln Phe Asn Arg Tyr Ala Ala Met Ala Leu
85 90 95

35

Lys Gly Leu Phe Asp Gln Leu Gly Leu Gly Arg Val Pro Leu Val Gly
100 105 110

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Asn Ser Leu Gly Gly Gly Thr Ala Val Arg Phe Ala Leu Asp Tyr Pro
115 120 125

Ala Arg Ala Gly Arg Leu Val Leu Met Gly Pro Gly Gly Leu Ser Ile
130 135 140

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Asn Leu Phe Ala Pro Asp Pro Thr Glu Gly Val Lys Arg Leu Ser Lys
145 150 155 160

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Phe Ser Val Ala Pro Thr Arg Glu Asn Leu Glu Ala Phe Leu Arg Val
165 170 175

Met Val Tyr Asp Lys Asn Leu Ile Thr Pro Glu Leu Val Asp Gln Arg
180 185 190

55

Phe Ala Leu Ala Ser Thr Pro Glu Ser Leu Thr Ala Thr Arg Ala Met

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 35 40 45
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 25 Arg Glu Trp Leu Arg Arg Ala Gln Glu Phe Glu Arg Ala Ala Gly Arg
 65 70 75 80
 30 Val Arg Gly Gln Arg Trp Gly Pro Arg Asn Leu Asp Val Asp Leu Ile
 85 90 95
 Ala Cys Tyr Gln Thr Ser Ala Thr Glu Ala Leu Val Glu Val Thr Ala
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 115 120 125
 40 Phe Val Leu Ile Pro Trp Ile Ala Val Asp Pro Thr Ala Gln Leu Thr
 130 135 140
 Val Ala Gly Cys Pro Arg Pro Val Thr Arg Leu Leu Ala Glu Leu Glu
 145 150 155 160
 45 Pro Ala Asp Arg Asp Ser Val Arg Leu Phe Arg Pro Ser Phe Asp Leu
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 180 185
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 55 <211> 564

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<212> DNA

<213> Mycobacterium tuberculosis

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<212> PRT

<213> Mycobacterium tuberculosis

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Cys Arg Pro Ala Asp Arg Ile Glu Ala Asp Thr Ala Ala Met Leu Thr
35 40 45
Leu Pro Pro Val Gly Pro Ser Ile Gly Trp Arg Thr Ser Thr Arg Leu
50 55 60
Pro Arg Asp His Tyr Val Arg Leu Asp Gly Asn Asp Tyr Ser Val His
65 70 75 80
Pro Val Ala Ile Gly Arg Arg Ile Glu Ile Thr Ala Asp Leu Ser Arg
85 90 95
Val Arg Val Trp Cys Gly Gly Thr Leu Val Ala Asp His Asp Arg Ile
100 105 110

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45

50

55

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Trp Ala Lys His Gln Thr Ile Ser Asp Pro Glu His Val Val Ala Ala
115 120 125

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Lys Leu Leu Arg Arg Lys Arg Phe Asp Ile Val Gly Pro Pro His His
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Val Glu Val Glu Gln Arg Leu Leu Thr Thr Tyr Asp Thr Val Leu Gly
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Leu Asp Gly Pro Val Ala
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<210> 270

<211> 498

<212> DNA

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<213> Mycobacterium tuberculosis

<400> 270

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<210> 271

<211> 350

<212> PRT

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<213> Mycobacterium tuberculosis

<400> 271

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Arg Ala Gln Pro Arg Leu Ala Glu Gln Leu Glu His Gly Gly Leu Arg
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 Leu Arg Arg Arg Ser Val Leu Ala Ser Ala Ala Arg Arg Val Leu Gly
 325 330 335
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 <212> DNA
 <213> Mycobacterium tuberculosis
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 <212> PRT

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<213> Mycobacterium tuberculosis

5

<400> 273

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20 25 30

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Glu Asp Leu Gly Trp His Thr Ile Cys Ala Val Met Thr Thr Glu Phe
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Leu Ala His Gln Arg Ser Val Gly Asn Asp Leu Ser Ile Ala Arg Pro
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20

Pro Arg Trp Leu Arg Pro
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25

<210> 274

<211> 210

<212> DNA

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<213> Mycobacterium tuberculosis

<400> 274

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<210> 275

<211> 846

<212> PRT

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<213> Mycobacterium tuberculosis

<400> 275

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55

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20 25 30

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Thr Ala Ala Leu Ala Gly Cys Ala Gly Met Ala Gly Asp Asp Pro Ala
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 5
 Gly Ala Val Phe Gly Arg Ser Tyr Asp Gly Ser Ala Ala Ala Leu Val
 50 55 60
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 Gln Ala Met Ser Val Ala Arg Asn Gly Leu Cys Asn Leu Gly Asp Gly
 65 70 75 80
 Val Arg Met Ser Ala His Asn Tyr Ser Leu Ala Glu Ala Met Ser Asp
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 Val Ala Gly Arg Ala Ala Pro Leu Pro Ala Pro Pro Pro Ser Gly Cys
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 Val Gly Val Gly Ala Pro Pro Ser Ala Val Gly Gly Gly Gly Gly Ala
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 Asn Gly Asp Ser Thr Lys Leu Arg Ala Ala Ala Val Ala Trp Arg Ser
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 Cys His Gln Leu Ala Ala Gln Leu Asp Ala Tyr Ala Ala Arg Ile Asp
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 Phe Ser Gly Glu Val Asp Ala Leu Ala Ala Glu Ile Thr Ala Val Val
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 Ser His Ala Glu Ala Val Ile Thr Ala Met Ala Asp His Ala Gly Lys
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 20 Lys Gln Phe Gly Lys Ser Leu Ile His Trp Asp Glu Trp Thr Thr Asn
 385 390 395 400
 25 Pro Asn Glu Ala Leu Gly Lys Thr Val Phe Asp Ala Ala Thr Leu Ala
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 420 425 430
 30 Leu Ala Gly Val Arg Gly Leu Lys Glu Arg Leu Glu Pro Thr Thr Pro
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 40 Lys Pro Ala Pro Val Pro Ala Asn Gly Pro Leu Pro His Ser Pro Thr
 485 490 495
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 50 Gly Thr His Val Pro His Gly Leu Pro Gln Pro Gly Glu His Val Pro
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 545 550 555 560
 55 Ser Ala Pro Ala Thr Ala His Gln Pro Gln Trp Ala Thr Thr Pro Ala

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			755					760					765			
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<213> Mycobacterium tuberculosis

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	Ala Leu Ala Leu Arg Ala Met Ala Pro Thr Gln Leu Ala Ala Ala Val	
	35 40 45	
50	Met Leu Ala Ala Gly Gly Val Val Ala Val Ala Ala Pro Gly His Thr	
	50 55 60	
	Ala Leu Met Val Val Ile Val Cys Ile Ala Gly Ala Val Gly Thr Leu	
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Ala Cys His
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15 <212> DNA
<213> Mycobacterium tuberculosis

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30 <210> 279
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35 <212> PRT
<213> Mycobacterium tuberculosis

40 <400> 279
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Ser Gly Phe Glu Val Ala Thr Ala Val Asp Gly Ala Glu Ala Leu Arg
35 40 45

50 Ser Ala Thr Glu Asn Arg Pro Asp Ala Ile Val Leu Asp Ile Asn Met
50 55 60

55 Pro Val Leu Asp Gly Val Ser Val Val Thr Ala Leu Arg Ala Met Asp
65 70 75 80

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Asn Asp Val Pro Val Cys Val Leu Ser Ala Arg Ser Ser Val Asp Asp
 85 90 95
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 Arg Val Ala Gly Leu Glu Ala Gly Ala Asp Asp Tyr Leu Val Lys Pro
 100 105 110
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 Phe Val Leu Ala Glu Leu Val Ala Arg Val Lys Ala Leu Leu Arg Arg
 115 120 125
 Arg Gly Ser Thr Ala Thr Ser Ser Ser Glu Thr Ile Thr Val Gly Pro
 130 135 140
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 Leu Glu Val Asp Ile Pro Gly Arg Arg Ala Arg Val Asn Gly Val Asp
 145 150 155 160
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 Val Asp Leu Thr Lys Arg Glu Phe Asp Leu Leu Ala Val Leu Ala Glu
 165 170 175
 His Lys Thr Ala Val Leu Ser Arg Ala Gln Leu Leu Glu Leu Val Trp
 180 185 190
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 Gly Tyr Asp Phe Ala Ala Asp Thr Asn Val Val Asp Val Phe Ile Gly
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<211> 112

<212> PRT

15 <213> Mycobacterium tuberculosis

<400> 281

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25 Val Ser Leu Gly Tyr Leu Ser Glu Ile Glu Arg Gly Arg Lys Glu Pro
35 40 45

30 Ser Ser Glu Leu Leu Ser Ala Ile Cys Thr Ala Leu Gln Leu Pro Leu
50 55 60

Ser Val Val Leu Ile Asp Ala Gly Glu Arg Met Ala Arg Gln Glu Arg
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35 Leu Ala Arg Ala Thr Pro Ala Gly Arg Ala Thr Gly Ala Thr Ile Asp
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40 Ala Ser Thr Lys Val Val Ile Ala Pro Val Val Ser Leu Ala Val Ala
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<210> 282

45 <211> 336

<212> DNA

<213> Mycobacterium tuberculosis

50 <400> 282

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55 atcgagcgcg gtcgcaagga gccttcacgc gagctgctca gtgcgatttg tacggctctg 180

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Claims

1. A therapeutic agent for combating mycobacterial infections, comprising an *M. tuberculosis* peptide having the sequence SEQ ID NO: 125, or a variant thereof having at least 70% amino acid identity therewith, or a fragment thereof having at least 10 amino acid residues, or a derivative thereof, wherein said variant, fragment or derivative has a common antigenic cross-reactivity to said isolated peptide, wherein the peptide is encoded by an *M. tuberculosis* gene the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium.
2. An inhibitor of an *M. tuberculosis* peptide having the sequence SEQ ID NO: 125, wherein the peptide is encoded by an *M. tuberculosis* gene the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium; wherein the inhibitor is an antisense or triplex-forming nucleic acid sequence which is complementary to at least part of the inducible or up-regulatable gene; and wherein the inhibitor is capable of inhibiting the *M. tuberculosis* peptide from exerting its native biological effect.
3. An antibody which binds to an *M. tuberculosis* peptide having the sequence SEQ ID NO: 125, or a variant thereof having at least 70% amino acid identity therewith, or a fragment thereof having at least 10 amino acid residues, or a derivative thereof, wherein said variant, fragment or derivative has a common antigenic cross-reactivity to said isolated peptide, wherein the peptide is encoded by a gene, the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium.
4. An attenuated *M. tuberculosis* mycobacterium in which a gene has been modified thereby rendering the mycobacterium substantially non-pathogenic, wherein the expression of said gene is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium, wherein the *M. tuberculosis* gene has a wild-type coding sequence of SEQ ID NO: 126.
5. An attenuated microbial carrier, comprising an *M. tuberculosis* peptide having the sequence SEQ ID NO: 125, or a variant thereof having at least 70% amino acid identity therewith, or a fragment thereof having at least 10 amino acid residues, or a derivative thereof, wherein said variant, fragment or derivative has a common antigenic cross-reactivity to said isolated peptide, wherein said peptide is encoded by an *M. tuberculosis* gene, the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium.
6. An attenuated microbial carrier according to Claim 5, wherein the attenuated microbial carrier is attenuated salmonella, attenuated vaccinia virus, attenuated fowlpox virus, or attenuated *M. bovis* (eg. BCG strain).

7. A DNA plasmid comprising a promoter, a polyadenylation signal, and a DNA sequence of SEQ ID NO: 126, or a variant thereof having at least 70% nucleotide sequence identity therewith, or a fragment thereof having at least 15 nucleotides, or a derivative thereof, wherein the peptide encoded by said variant, fragment or derivative has a common antigenic cross-reactivity to the peptide encoded by said DNA sequence,
 wherein said DNA sequence is the coding sequence of an *M. tuberculosis* gene, the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium; and
 wherein the promoter and polyadenylation signal are operably linked to the DNA sequence.
8. A DNA plasmid according to Claim 7, wherein the promoter is selected from the group consisting of CMV and SV40 promoters, and/or the polyadenylation signal is selected from SV40 and bovine growth hormone polyadenylation signals.
9. An isolated RNA sequence that is encoded by a DNA sequence of SEQ ID NO: 126, or a variant thereof having at least 70% nucleotide sequence identity therewith, or a fragment thereof having at least 15 nucleotides, or a derivative thereof, wherein the peptide encoded by said variant, fragment or derivative has a common antigenic cross-reactivity to the peptide encoded by said DNA sequence,
 wherein the DNA sequence is the coding sequence of an *M. tuberculosis* gene which is induced or up-regulated during culture of an *M. tuberculosis* mycobacterium under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium.
10. An RNA vector comprising the RNA sequence of Claim 9 and an integration site for a chromosome of a host cell.
11. Use of an *M. tuberculosis* peptide having the sequence SEQ ID NO: 125, or a variant thereof having at least 70% amino acid identity therewith, or a fragment thereof having at least 10 amino acid residues, or a derivative thereof, wherein said variant, fragment or derivative has a common antigenic cross-reactivity to said isolated peptide, wherein the peptide is encoded by an *M. tuberculosis* gene the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium;
 an inhibitor according to Claim 2;
 an antibody according to Claim 3;
 an attenuated *M. tuberculosis* mycobacterium according to Claim 4;
 an attenuated microbial carrier according to Claim 5 or 6;
 a DNA sequence of SEQ ID NO: 126, or a variant thereof having at least 70% nucleotide sequence identity therewith, or a fragment thereof having at least 15 nucleotides, or a derivative thereof, wherein the peptide encoded by said variant, fragment or derivative has a common antigenic cross-reactivity to the peptide encoded by said DNA sequence, wherein said DNA sequence is the coding sequence of an *M. tuberculosis* gene which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium;
 a DNA plasmid according to Claim 7 or 8;
 an RNA sequence according to Claim 9; and/or
 an RNA vector according to Claim 10;
 for the manufacture of a medicament for treating or preventing a mycobacterial infection.
12. Use of an *M. tuberculosis* peptide having the sequence SEQ ID NO: 125, or a variant thereof having at least 70% amino acid identity therewith, or a fragment thereof having at least 10 amino acid residues, or a derivative thereof, wherein said variant, fragment or derivative has a common antigenic cross-reactivity to said isolated peptide, wherein the peptide is encoded by an *M. tuberculosis* gene the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium;

an antibody according to Claim 3; or

a polynucleotide probe comprising at least 8 nucleotides wherein said probe binds to at least part of an *M. tuberculosis* gene which is encoded by a DNA sequence of SEQ ID NO: 126, or a variant thereof having at least 70% nucleotide sequence identity therewith, or a fragment thereof having at least 15 nucleotides, or a derivative thereof, wherein the peptide encoded by said variant, derivative or fragment has a common antigenic cross-reactivity to the peptide encoded by said gene, wherein said gene is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium;
for the manufacture of a diagnostic reagent for identifying a mycobacterial infection.

13. An *M. tuberculosis* peptide having the sequence SEQ ID NO: 125, or a variant thereof having at least 70% amino acid identity therewith, or a fragment thereof having at least 10 amino acid residues, or a derivative thereof, wherein said variant, fragment or derivative has a common antigenic cross-reactivity to said isolated peptide, wherein the peptide is encoded by an *M. tuberculosis* gene the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium;
an inhibitor according to Claim 2;
an antibody according to Claim 3;
an attenuated *M. tuberculosis* mycobacterium according to Claim 4;
an attenuated microbial carrier according to Claim 5 or 6;
a DNA sequence of SEQ ID NO: 126, or a variant thereof having at least 70% nucleotide sequence identity therewith, or a fragment thereof having at least 15 nucleotides, or a derivative thereof, wherein the peptide encoded by said variant, fragment or derivative has a common antigenic cross-reactivity to the peptide encoded by said DNA sequence, wherein said DNA sequence is the coding sequence of an *M. tuberculosis* gene which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium;
a DNA plasmid according to Claim 7 or 8;
an RNA sequence according to Claim 9; and/or
an RNA vector according to Claim 10;
for use in treating or preventing a mycobacterial infection.
14. An *M. tuberculosis* peptide having the sequence SEQ ID NO: 125, or a variant thereof having at least 70% amino acid identity therewith, or a fragment thereof having at least 10 amino acid residues, or a derivative thereof, wherein said variant, fragment or derivative has a common antigenic cross-reactivity to said isolated peptide, wherein the peptide is encoded by an *M. tuberculosis* gene the expression of which is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium;
an antibody according to Claim 3; or
a polynucleotide probe comprising at least 8 nucleotides wherein said probe binds to at least part of an *M. tuberculosis* gene which is encoded by a DNA sequence of SEQ ID NO: 126, or a variant thereof having at least 70% nucleotide sequence identity therewith, or a fragment thereof having at least 15 nucleotides, or a derivative thereof, wherein the peptide encoded by said variant, derivative or fragment has a common antigenic cross-reactivity to the peptide encoded by said gene, wherein said gene is induced or up-regulated under culture conditions that are nutrient-starving and which maintain mycobacterial latency, said conditions being obtainable by batch fermentation of an *M. tuberculosis* mycobacterium for at least 40 days post-inoculation, when compared with culture conditions that are not nutrient-starving and which support exponential growth of said mycobacterium;
for use in identifying a mycobacterial infection.

Fig. 1

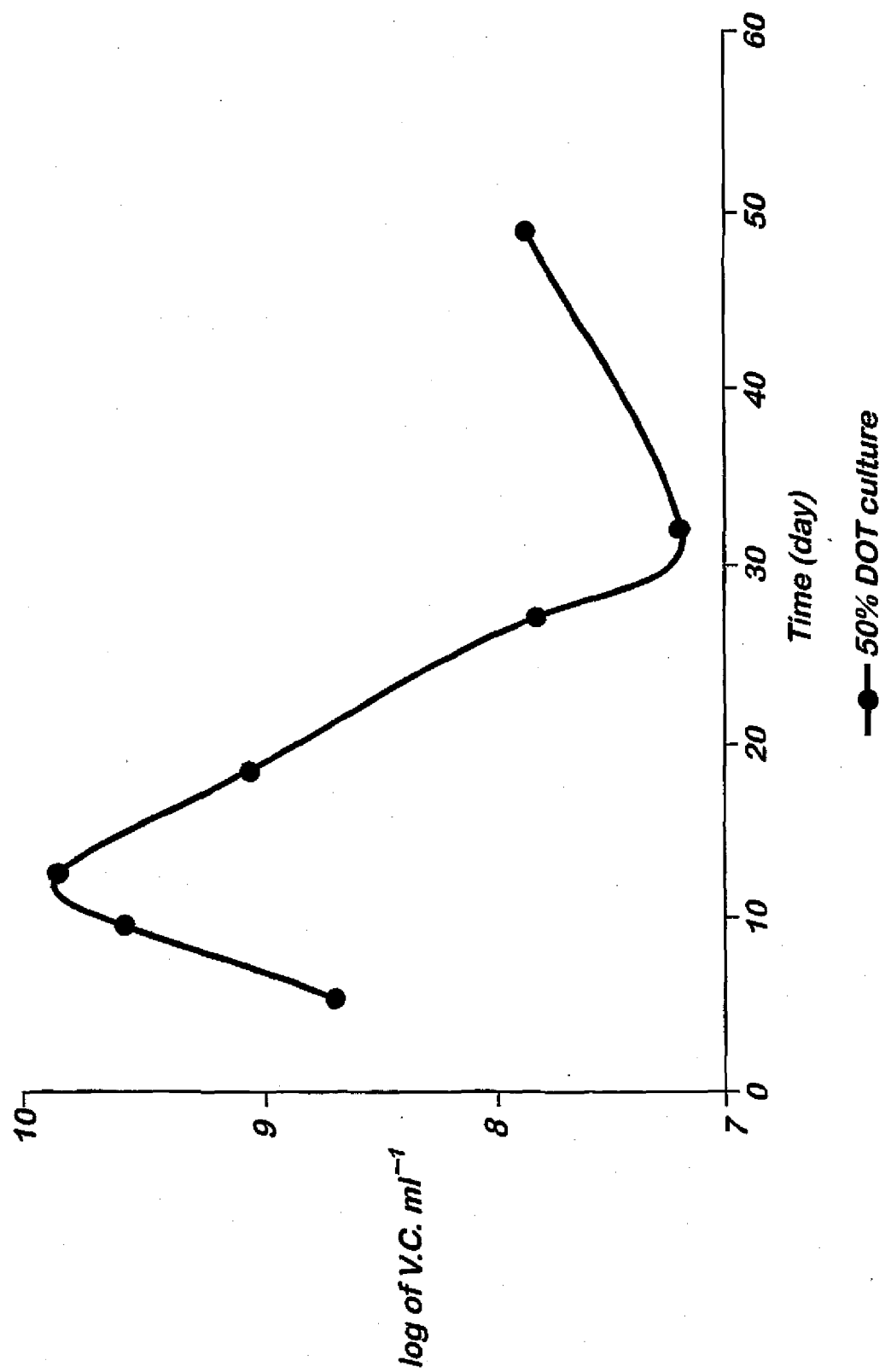


Fig. 2

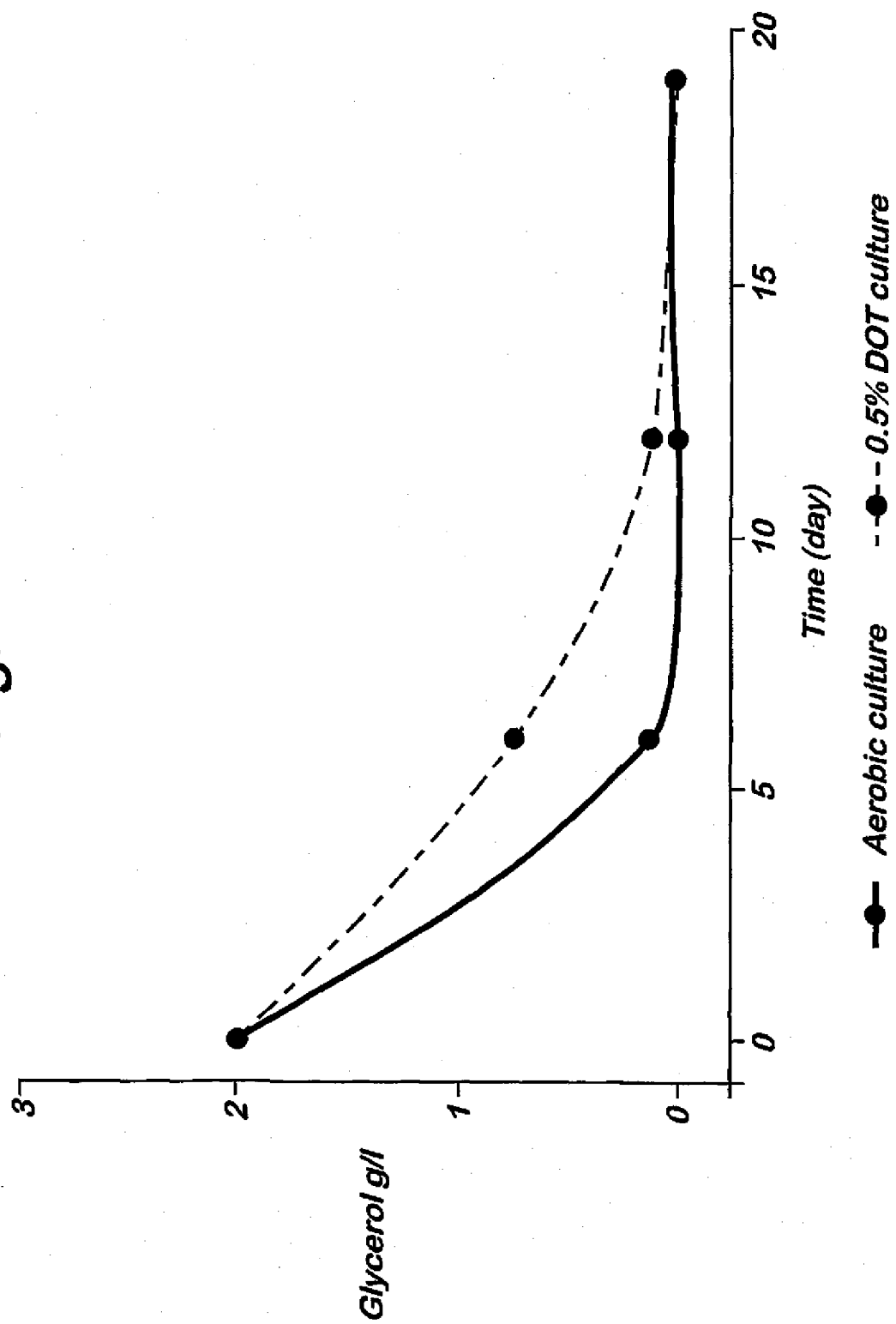


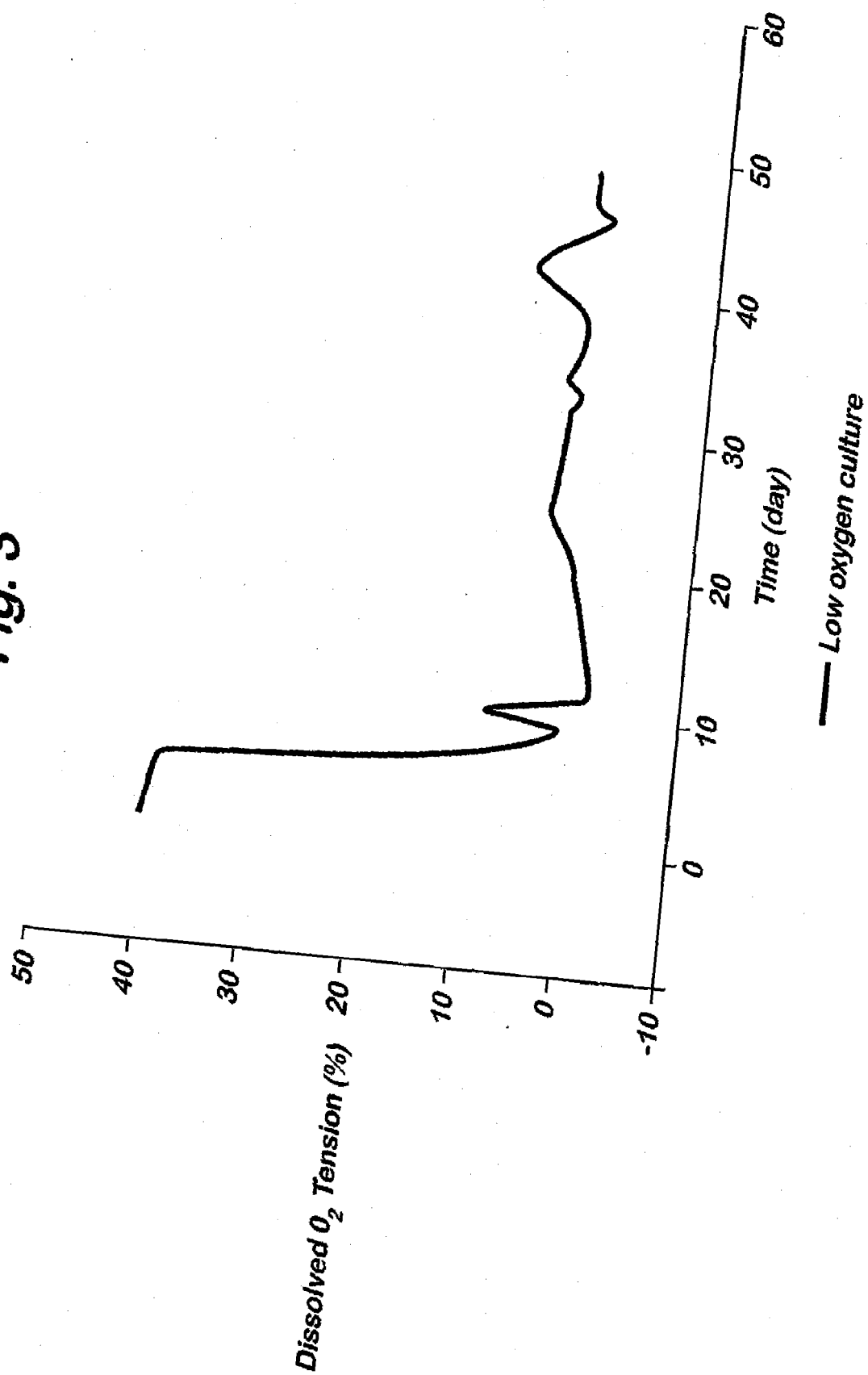
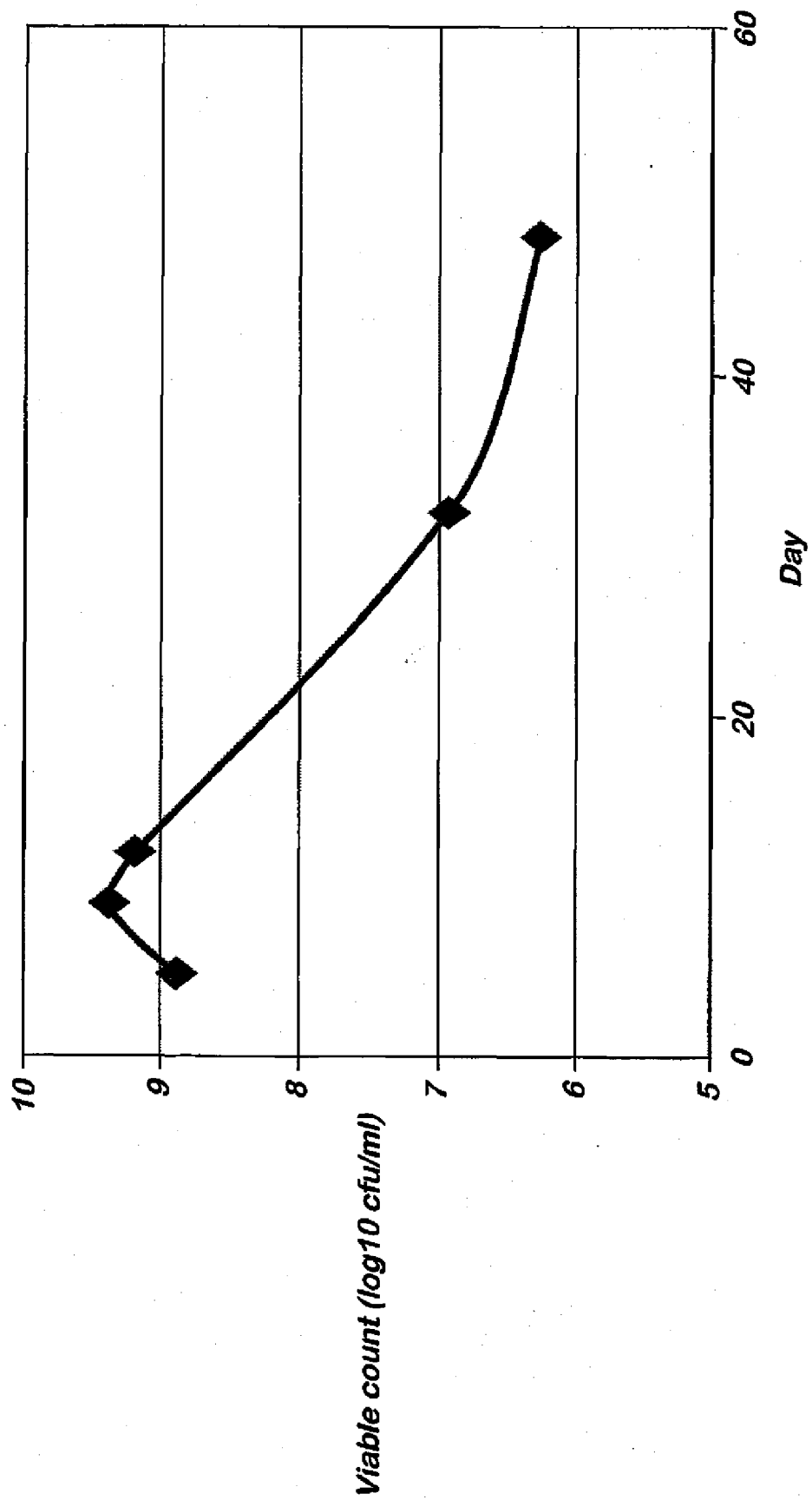
Fig. 3

Fig. 4





EUROPEAN SEARCH REPORT

Application Number
EP 10 15 0877

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	DATABASE Geneseq [Online] 24 March 1999 (1999-03-24), "M. tuberculosis antigen clone Tb436 protein sequence." XP002415392 retrieved from EBI accession no. GSN:AAW73663 Database accession no. AAW73663 * the sequence *	1-14	INV. C07K14/35 C12N15/31 C12N15/74 C07K16/12 G01N33/50 C12N15/11 A61K39/00 C12N15/86 C12N5/10 A61K38/16
A	& WO 98/53076 A2 (CORIXA CORP [US]) 26 November 1998 (1998-11-26) -----	1	
A	MAHENTHIRALINGAM E ET AL: "CLONING AND SEQUENCING OF THE GENE WHICH ENCODES THE HIGHLY INDUCIBLE ACETAMIDASE OF MYCOBACTERIUM SMEGMATIS" JOURNAL OF GENERAL MICROBIOLOGY, SOCIETY FOR MICROBIOLOGY, READING, GB, vol. 139, 1993, pages 575-583, XP002929577 ISSN: 0022-1287 -----	1-14	
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The present search report has been drawn up for all claims			
Place of search Munich		Date of completion of the search 1 April 2010	Examiner Marinoni J-C
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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